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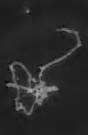

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THE INHERITANCE OF A MUTANT CHARACTER IN *PUCCINIA GRAMINIS TRITICI*[1]

BY T. JOHNSON[2] AND MARGARET NEWTON[2]

Abstract

A study was made of the inheritance of an abnormal characteristic of *Puccinia graminis Tritici* Erikss. and Henn. race 21, namely, the production on barberry of white haploid pustules that developed few or no pycnia and rarely produced aecia but occasionally gave rise to uredia and telia. In this race, approximately 50% of the pustules were white, the remainder normal. By selfing studies and by crosses with another physiologic race, it was demonstrated that diploidisation of the mycelia of normal pustules by pycniospores from white pustules initiated physiologic races that produced white and normal pustules, on the barberry, in approximately equal numbers, whereas normal × normal matings produced normal rust and white × white matings were sterile. Uredia that occasionally arose in white pustules as a result of diploidisation by either pycniospores or mycelia of normal pustules gave rise to physiologic races producing white and normal pustules in about equal numbers. The capacity to develop white pustules is not confined to any one sex and is not limited to any particular physiologic race. It is assumed that a mutation affecting one of the conjugate nuclei took place in the original culture of race 21 and that, during meiotic divisions in the germinating teliospore, the mutant factor is segregated so that half of the sporidia give rise to white and half to normal pustules.

Introduction

A number of abnormal characteristics of *Puccinia graminis Tritici* Erikss. and Henn. ascribable to mutation have been reported. These include atypical colour of urediospores (5, 8), teliospores, and aeciospores (7), decrease in vigour of uredial production, and impairment of the ability to produce aecia, with which is associated occasionally a limited ability to develop uredia and telia on the barberry (3, 6). In other varieties of *Puccinia graminis* Pers., abnormal characteristics have been reported by Gordon (2), Cotter (1), and Johnson and Newton (4).

In the present paper is reported a study of the inheritance of an abnormal characteristic originally encountered in a culture of race 21 of wheat stem rust collected at Indian Head, Sask., in 1934, namely, the production on barberry of white haploid pustules (6). When barberries were inoculated with this race, haploid pustules of two different kinds were produced (Fig. 2). Pustules of the one kind were normal in appearance and contained numerous

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Contribution No. 732 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Plant Pathologist and Senior Plant Pathologist, respectively, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

pycnia with abundant nectar the intermixing of which was followed by the development of aecia. Pustules of the other kind were white in colour, and developed either no pycnia or only a few scattered ones after a period of several weeks. When pycnia were formed in the white pustules, they frequently produced a moderate quantity of yellow nectar which, when applied to pustules of the normal kind, led to the formation of aecia. When the nectar of white pustules, however, was intermixed, no aecial formation resulted. The application of nectar of normal pustules to white pustules produced either no aecia or more or less abnormal ones that rarely grew to full development.

Casual inspection of infected barberry plants indicated that the two kinds of pustule were present in approximately equal numbers, but, when counts of the normal and white pustules were made, it was found that on almost every barberry plant the normal pustules were somewhat more numerous (Fig. 1). The disparity in number between the two types of pustule was so small as to suggest that sporidia capable of giving rise to the two pustule types were produced in equal numbers but that the infectivity of the sporidia that gave rise to the normal pustules was the higher of the two.

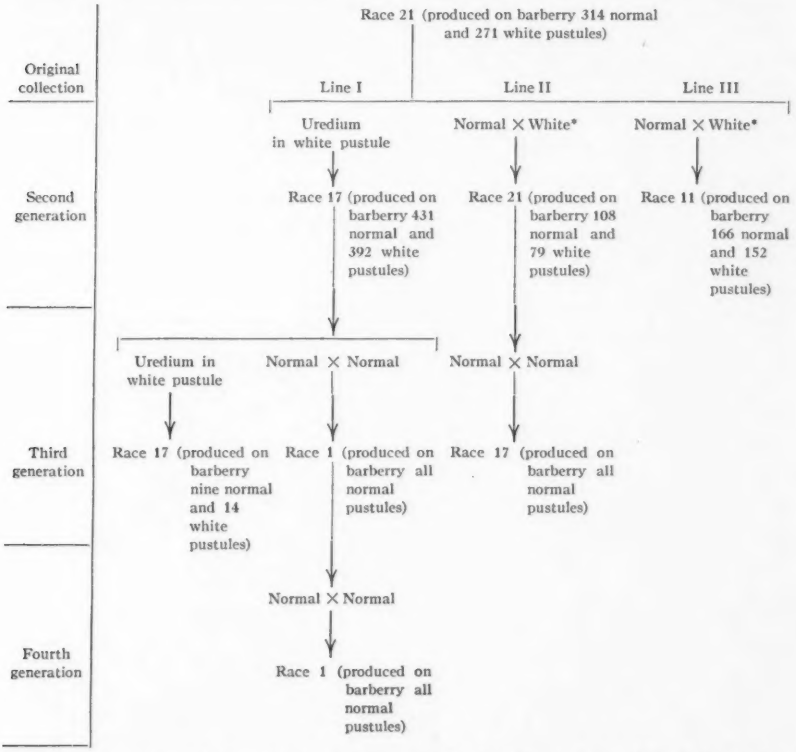
The production of aecia in normal pustules as a result of the application to them of nectar from white pustules raised the question of whether the next generation of rust arising from such aecia would produce both types of pustule. An attempt to answer this question formed the beginning of the progeny studies here reported.

Progeny Studies with Race 21

In the progeny studies undertaken to elucidate the inheritance of the above-mentioned abnormality, nectar was transferred from normal to normal pustules, from normal to white, from white to normal, and from white to white, with the object of securing aecia that could be used to originate uredial cultures derived from all possible nuclear associations. Aecia developed readily in normal pustules as a result of the application to them of nectar from either normal or white pustules; but, in white pustules, the production of aecia was so rare that no uredial cultures originating from them could be established. Uredial progeny from white pustules was nevertheless obtained. As reported previously (3, 6), uredia were occasionally formed in white pustules on the barberry, usually after a prolonged period. Although the manner in which diploidisation occurred is not certain, it is likely that in most cases it resulted from either the fusion of the mycelium of a normal pustule with that of a white one or as a consequence of the transference of pycniospores from a normal pustule to a white one. The progeny studies include two uredial cultures that originated in white pustules by one or the other of these means. No uredial cultures were obtained from the fusion of the mycelia of white pustules or as a result of the intermixing of their nectar, and there is, in fact, no evidence that either aecia or uredia do originate by these means.

In Fig. 1 are summarized progeny studies with three lines of rust derived from race 21 collected at Indian Head, Sask. Line I originated on barberry

in a white pustule containing a single uredium that gave rise to a pure culture of race 17. Teliospores of this culture were used to inoculate several barberries on which were produced 431 normal and 392 white pustules. On one of these barberry plants, a transfer of nectar from a normal to a normal pustule produced aecia that gave rise to a culture of race 1. This race has produced only normal pustules for two successive generations. On another of the barberry plants inoculated with race 17, a uredium was formed in a white pustule.



* *Aecia were formed in a normal pustule as a result of application to it of nectar from white pustules.*

FIG. 1. *Progeny studies with race 21 from Indian Head, Sask.*

This initiated a culture of race 17 that produced both normal and white pustules on barberry. In lines II and III, normal × white matings produced races that gave rise to both types of pustule, whereas a normal × normal mating in line II originated a normal culture of race 17.

The progeny studies show that the association of nuclei from normal and white pustules results in the formation of a strain of rust that is capable of producing both types of pustule on barberry. On the other hand, the associa-

tion of nuclei from two normal pustules leads to the production of a strain of rust that is entirely free from the presence of white pustules. If a strain of rust resulting from the association of nuclei from two white pustules could have been established, it would presumably have produced, on barberry, white pustules only.

Crosses Involving the Transfer of Nectar from a White Pustule to Haploid Pustules of Another Physiologic Race

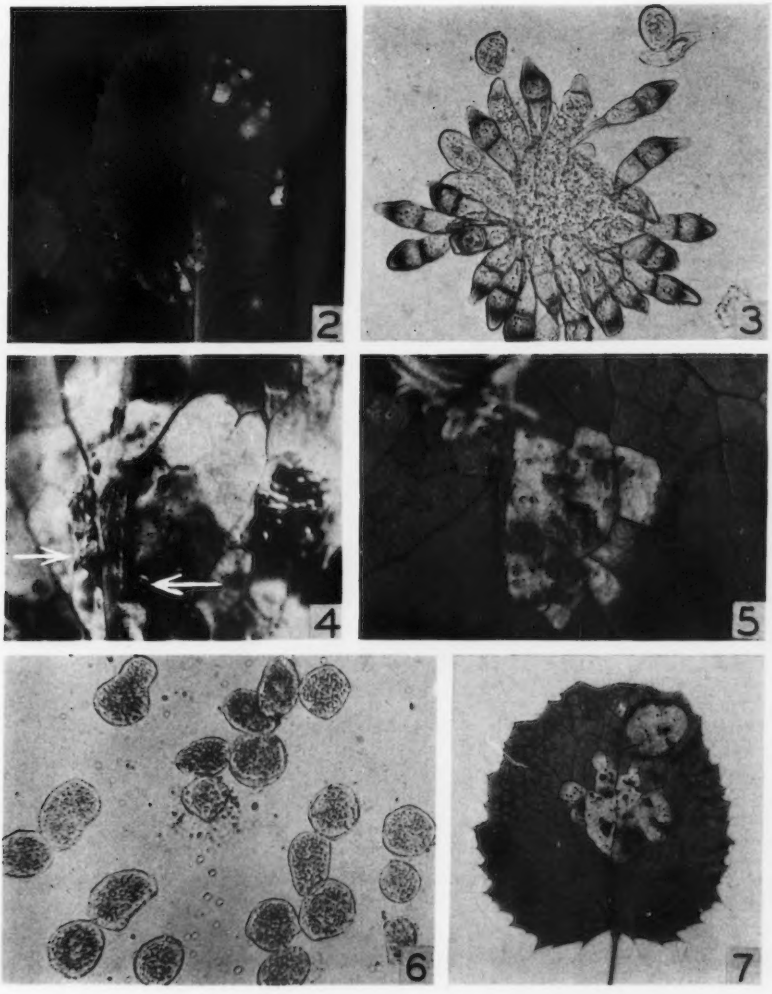
In the progeny studies with race 21, the approximately equal distribution of white and normal pustules had suggested a one to one ratio which indicated the presence of a single mutant factor. The studies had also shown that the capacity to produce white haploid pustules on barberry was not necessarily confined to that race as its progeny contained cultures of races 11 and 17 that showed the same characteristic. There was therefore no apparent reason why the ability to produce white pustules should not be transmitted to other physiologic races by means of crossing, i.e., the transference of the nectar of white pustules to normal haploid pustules of other races. The resulting aeciospores and their uredial offspring should contain one nucleus bearing a factor for the production of white pustules and another bearing a factor for the production of normal pustules; the teliospores should germinate to produce sporidia of which approximately one-half would give rise to white pustules on the barberry.

To test this supposition, nectar was transferred from one white pustule of race 17, a race derived from the selfing of race 21, to nine presumably haploid pustules of race 152, a race producing only normal pustules. Aecia were produced in three of these pustules and uredial cultures were established from them. The uredial progeny from two of the pustules was identified as race 48, but one pustule produced two races, namely, races 48 and 21. Telial material was obtained from the culture of race 21 and from one of the cultures of race 48, and infection of barberry was secured with both. Race 21 produced on barberry 87 normal and 91 white pustules, and race 48 produced 158 normal and 148 white pustules. The appearance of white pustules in the progeny of the crosses shows that the ability to produce white pustules is readily transmissible to other races by diploidisation through the instrumentality of the pycniospores, and the approximately equal distribution of white and normal pustules supports the assumption that the production of white pustules is governed by a single mutant factor.

The Production of Pycnia, Aecia, and Uredia in White Pustules

As no detailed description of the white pustules produced by race 21 and its progeny was included in earlier reports (3, 6), it seems advisable to record here some of their chief characteristics. They first become visible a few days after inoculation as white necrotic flecks and are, from their first appearance, easily distinguishable by their colour from normal pustules (Fig. 2). The white colour is partly due to the absence of pycnial nectar but chiefly to chlorophyll

PLATE I



EXPLANATION OF FIGURES

FIG. 2. *White and normal pustules of race 21 on a barberry leaf 23 days after inoculation.*

FIG. 3. *Urediospores and teliospores formed in a white pustule.*

FIGS. 4 AND 5. *Caeoma-like structures of aeciospores on the lower surfaces of white pustules, and normal aecia present in adjacent normal pustules.*

FIG. 6. *Aeciospores from one of the caeoma-like structures.*

FIG. 7. *Lower surface of a barberry leaf showing a compound pustule of race 21, one component of which contains aecia, the other, small uredia.*

destruction in the infected area, which is much more pronounced than in normal infections. Sooner or later many of the white pustules develop pycnia and nectar. Of 172 pustules that were examined periodically, 105 eventually formed pycnia while 67 did not. Pycnia, however, were never as numerous in white as in normal pustules. A comparison of the abundance of pycnial production in the two types of pustule was obtained by counting the pycnia in two normal and three white pustules 23 days after inoculation. The two normal pustules bore respectively 92 and 91 pycnia whereas the three white pustules bore respectively 0, 1, and 10 pycnia. Proto-aecia were numerous in the normal pustules but none had formed in the white.

The development of aecia in white pustules was rare and nearly always limited to rudimentary or otherwise atypical structures. Out of 1091 white pustules that were kept under observation only 20 formed aeciospores. Of these, 13 produced the spores in structures resembling caeoma but surrounded by a peridium (Figs. 4 and 5) which enclosed short chains of rather irregularly shaped spores of which many were colourless and apparently dead (Fig. 6). Most of this aecial development took place only after a long period—the earliest observed formation of aecia being 38 days after inoculation.

Uredia were formed in white pustules somewhat more frequently than aecia, but nevertheless in only a small proportion of the pustules. Out of the 1091 white pustules mentioned above only 29 produced uredia. In many of these pustules teliospores as well as urediospores were present (Fig. 3). In one instance, urediospores and teliospores were produced in a white pustule that previously had formed a caeoma-like structure of aeciospores. The uredia, like the aecia, were formed only in white pustules that had reached considerable age. The urediospores were incapable of infecting barberries.

Discussion

In cultures of race 21 and its derivatives that produced both normal and white haploid pustules, it was found that out of a total of 2420 pustules, 1273 (52.6%) were normal and 1147 (47.4%) were white. These data would indicate that approximately 50% of the sporidia produced by the germinating teliospores carried the factor governing the production of white pustules. The slightly lower number of white pustules is likely the result of a somewhat lower infectivity on the part of the sporidia producing them. The presence of white pustules in the original culture of race 21 collected at Indian Head, Sask., may be accounted for by assuming that a mutation had occurred in this culture. As half of the sporidia were affected, the mutant factor was presumably located in one of the two associated urediospore nuclei. It is evident that both sexes are represented in normal pustules as the intermixing of their nectar leads to aeciospore formation. The mutant factor is therefore not closely associated with the sex factor and is either located in a different chromosome or in a locus so distantly removed from the sex factor as to allow crossing over. Neither does the mutant factor appear to be limited to any particular physiologic race, for, although it originated in physiologic race 21,

it was introduced by selfing and crossing into three other physiologic races, namely, races 11, 17, and 48. It appears, therefore, that the mutant character is not confined to any one sex or any particular physiologic race.

The effect of the mutant factor is manifested by a marked destruction of chlorophyll in the infected area, and a retardation of the growth of pycnia as well as a reduction in their number. The production of pycnial nectar is therefore delayed and its quantity reduced. Proto-aecia are sometimes initiated and are capable of limited aeciospore production as a result of the application of nectar of normal pustules. Uredia occasionally arise in white pustules through the action of either the pycniospores or the mycelia of normal pustules. There is no evidence that the intermixing of the nectar of white pustules produces either aecia or uredia. The pycniospores of white pustules, however, are functional as their application to normal pustules leads to aecial formation. The mutant factor resembles the well known lethal factors of higher plants in that it appears to be lethal to the rust when present in a homozygous condition but does not notably affect its survival value in the heterozygous state.

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A CONTRIBUTION TOWARD A CLARIFICATION OF THE *TRAMETES SERIALIS* COMPLEX[1]

By Mildred K. Nobles[2]

Abstract

The fungus frequently isolated from a destructive, brown, cubical rot in Sitka spruce and Douglas fir, and formerly referred to *Trametes serialis* Fries, has been connected with a fruit body collected on Sitka spruce in the Queen Charlotte Islands, B.C. By means of morphological, cultural, and interfertility studies it has been shown to be distinct from *Trametes serialis*, and is described herewith under the name *Poria microspora* Overholts, n. sp. Similar studies of *Poria Sequoiae* Bonar, *Polyporus palustris* Berk., and *Poria carbonica* Overholts, n. sp., all of which have been confused with *Trametes serialis* because of similarities between fruit bodies or cultures, have demonstrated the validity of each of the species and have provided criteria for their separation on the basis of cultural characters.

Introduction

Forest and timber pathologists have long been familiar with a fungus frequently isolated from a brown, cubical rot that occurs in coniferous wood in service and, more rarely, in living trees. Concerning this fungus Cartwright (6) has written that it is a common cause of brown pocket rot in Sitka spruce timber imported into Great Britain from Canada, and Cartwright and Findlay (7) have stated that it is "quite commonly found in imported Canadian timber, and is undoubtedly the most important cause of decay ("dote") in imported Douglas fir". Up to the present this important organism has been known only from cultures isolated from rot and has had, therefore, to be identified on the basis of its cultural characters. This was done by Cartwright (6), who set forth apparently conclusive evidence in support of its being *Trametes serialis* Fries, under which name it has been known during the intervening years. Unfortunately the cultures of *T. serialis* obtained and used by Cartwright for comparison had been incorrectly identified and therefore the name *T. serialis* was applied erroneously to this fungus. That the fungus is not *T. serialis* has been recognized by Cartwright (Letter May 11, 1942), Davidson (Letter September 2, 1942), and others for some time, but the name has been retained for convenience. As described later, this fungus has been connected with a fruit body collected from *Picea sitchensis* by Dr. J. E. Bier in the Queen Charlotte Islands off the coast of British Columbia. It appears to be an undescribed species and is accordingly described below under the name *Poria microspora* Overholts, n. sp.

Since the separation of this new species from *Trametes serialis* requires a knowledge of both species, cultural and morphological descriptions of each are included. *Polyporus palustris*, *Poria Sequoiae*, and *Poria carbonica* have

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Contribution No. 733 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Junior Plant Pathologist, Central Laboratory.*

also been confused with *T. serialis* because of similarities between fruit bodies or cultures, and therefore descriptions of these species are presented, together with evidence in support of the validity of each species and methods for their separation.

Cultural Studies

Table I contains a list of the 51 isolates of these species that have been studied in culture, under their accession numbers in the Dept. of Agr., Ottawa, Mycological Herbarium, with data as to whether the culture was obtained from spores (*S*), tissue of fruit body (*T*), or rot (*R*), the host and locality where collected, and other pertinent information.

TABLE I

Herb. No.	Source	Host	Locality	Where obtained
Poria microspora				
*F*347	*R*	*Picea sitchensis* timber	—	Isolated from specimen of rot received from E. J. Butler, Imperial Bur. Mycology England, June 2. 1925.
*F*458	*R*	*Picea sitchensis* tree showing heart rot	Queen Charlotte Islands, B.C.	Collected and cultured, I. Mounce, Sept. 26, 1925.
*F*1280	*R*	—	—	Rec'd from K. St. G. Cartwright, For. Prod. Res. Lab., Princes Risborough, England, Mar. 6, 1930, under name *T. serialis*.
*F*7640	*R*	*Pseudotsuga taxifolia*	Oregon, U.S.A.	Cultured by C. J. Humphrey. Rec'd from C. A. Richards (575) For. Prod. Lab., Madison, Wis., Nov. 1, 1937, under name *T. serialis*.
*F*8009	?	—	—	Bell Telephone Lab. Cult. No. *V*10. Rec'd from C. W. Fritz, For. Prod. Lab. Ottawa, Canada, Jan. 14, 1938.
8191[1]	*R*	*Pinus Banksiana* railway ties	—	Isolated and submitted by C. W. Fritz (308–4). Rec'd July 13, 1938.
9176	*R*	*Pseudotsuga taxifolia*	—	Isolated and submitted by W. P. K. Findlay, For. Prod. Res. Lab., Princes Risborough, England. Rec'd May 8, 1939.
9251	*R*	*Tsuga heterophylla* standing tree showing heart rot	Vancouver, B.C.	Collected by I. Mounce *et al.*, June 2, 1939.

[1] *Numbers up to 8100 in the collection of wood-inhabiting fungi in the Dept. of Agr., Ottawa, Mycol. Herb. are preceded by F, to distinguish them from the parallel series of numbers in the general collection.*

TABLE I—*Continued*

Herb. No.	Source	Host	Locality	Where obtained
Poria microspora—Concluded				
9429	*R*	*Pseudotsuga taxifolia* timber in building	Toronto, Ont.	Rec'd from C. W. Fritz, Oct. 25, 1939.
9511	*R*	*Pseudotsuga taxifolia* signal post	England	Isolated and submitted by W. P. K. Findlay, For. Prod. Res. Lab., Princes Risborough, England. Rec'd Nov. 14, 1939.
10724	*R* and *T*	*Picea sitchensis* top rot in standing tree	Queen Charlotte Islands, B.C.	Isolated and submitted by J. E. Bier. Rec'd June 27, 1942.
10726	*R*	*Picea sitchensis* top rot in standing tree	Queen Charlotte Islands, B.C.	Isolated and submitted by J. E. Bier. Rec'd June 27, 1942.
Trametes serialis Fries				
*F*6813	*T* and *R*	*Picea* sp.	Gaspé, P.Q.	Collected C. G. Riley, Aug., 1936.
*F*7353	*S*	*Picea excelsa*	Norway	Cultured and submitted by H. Robak. Rec'd May 5, 1937.
*F*7399	*S*	?*Pinus sylvestris* bridge balk	Norway	Cultured and submitted by H. Robak. Rec'd May 5, 1937.
*F*7400	*S*	?*Pinus sylvestris*	Norway	Cultured and submitted H. Robak. Rec'd April 15, 1937.
9519	*S* and *T*	*Picea mariana*—cut and piled 1932	Eagle Depot, P.Q.	Collected C. G. Riley, Nov. 16, 1939.
9520	*S*	*Picea mariana*—cut and piled 1933	Eagle Depot, P.Q.	Collected C. G. Riley, Nov. 16, 1939.
9522	*S* and *T*	*Picea glauca*—cut and piled 1933	Eagle Depot, P.Q.	Collected C. G. Riley, Nov. 16, 1939.
9523	*S* and *T*	*Picea mariana*—cut and piled 1932	Eagle Depot, P.Q.	Collected C. G. Riley, Nov. 16, 1939.
10230	*S*	Coniferous log—bridge support	Calumet, P.Q.	Collected M. K. Nobles, Sept. 16, 1941.
10234	*S*	Coniferous log—bridge support	Calumet, P.Q.	Collected J. W. Groves, Sept. 16, 1941.
10727	*T* and *R*	*Picea sitchensis*	Queen Charlotte Islands, B.C.	Collected and cultured J. E. Bier, May 28, 1942.
10728	*T* and *R*	*Picea sitchensis*	Queen Charlotte Islands, B.C.	Collected and cultured J. E. Bier, May 28, 1942.
10912	*R*	*Tsuga canadensis* log	Kane, Pa., U.S.A.	Collected W. A. Campbell, Aug. 15, 1936. Rec'd from R. W. Davidson (71205-*R*) Oct. 8, 1942.

TABLE I—*Continued*

Herb. No.	Source	Host	Locality	Where obtained
Trametes serialis Fries—Concluded				
10913	*R*	*Tsuga canadensis*	Kane, Pa., U.S.A.	Collected W. A. Campbell, and L. O. Overholts, Aug. 14, 1936. Rec'd from R. W. Davidson (71213-*R*) Oct. 8, 1942.
10914	*R*	*Prunus serotina*	Westline, Pa., U.S.A.	Collected R. W. Davidson, May 14, 1940. Rec'd from R. W. Davidson (94023-*R*), Oct. 8, 1942.
10916	*R*	*Acer rubrum* log	Egle's Mere, Pa., U.S.A.	Collected W. A. Campbell and L. O. Overholts, Sept. 10, 1936. Rec'd from R. W. Davidson (71265-*R*), Oct. 8, 1942.
10917	*R*	*Prunus serotina*—living tree	Kane, Pa., U.S.A.	Rec'd from R. W. Davidson, (101-22), Oct. 8, 1942.
10918	?	Mine supports	Northern Ontario	Collected and cultured C. W. Fritz (3). Rec'd from R. W. Davidson, Oct. 8, 1942.
Poria Sequoiae Bonar				
8755	*T*	*Sequoia sempervirens*	—	Isolated and submitted by Lee Bonar. Rec'd Dec. 23, 1938.
Polyporus palustris Berk. & Curt.				
*F*2045	*T*	*Pinus Taeda* stump	Bude, Miss., U.S.A.	Rec'd from L. O. Overholts (55456), Oct. 20, 1931.
*F*2964	*T*	—	—	Rec'd from L. O. Overholts (14841-*S*), Dec. 27, 1932, under name *T. serialis*[1].
*F*7641	*T*	—	—	Isolated by W. H. Snell. Rec'd from C. A. Richards (661) For. Prod. Lab., Madison, Wis., Nov. 1, 1937, under name *T. serialis*.
*F*7642	*S*	—	—	Isolated from spores from fruit body in culture. Rec'd from C. A. Richards (662), For. Prod. Lab., Madison, Wis., Nov. 1, 1937, under name *T. serialis*.

[1] *Dr. Overholts has recently re-examined this fruit body (14841) and has found it to be Trametes serialis. The culture bearing this number is Polyporus palustris. Therefore an error must have been made at the time of isolation or subsequent transferring. In spite of this discrepancy, the culture has been retained, because it had been used in many intercollection crosses before the error was discovered.*

TABLE I—*Concluded*

Herb. No.	Source	Host	Locality	Where obtained
Polyporus palustris Berk. & Curt.—Concluded				
10250	?	—	—	Rec'd from W. H. Snell (19*M*), Oct. 29, 1941, under name *T. serialis* (?).
10618	*T*	*Pinus* sp. stump	Hammond, La., U.S.A.	Cultured by R. W. Davidson (57010) 1932. Rec'd from him Sept. 3, 1942.
10620	*R*	*Pinus* sp. board	Washington, D.C., U.S.A.	Rec'd from R. W. Davidson (182*a*), Sept. 3, 1942.
Poria carbonica Overholts				
8215	*R*	*Pseudotsuga taxifolia*	Cowichan Lake For. Expt. Sta., B.C.	Collected I. Mounce, June 14, 1938.
8427	*T*	*Pseudotsuga taxifolia*	Royston, B.C.	Collected I. Mounce, July 29, 1938.
8248	*T*	*Pseudotsuga taxifolia*	Cameron Lake, B.C.	Collected I. Mounce, July 30, 1938.
8250	*T*	*Pseudotsuga taxifolia*	Cameron Lake, B.C.	Collected I. Mounce, July 30, 1938.
8254	*T*	*Pseudotsuga taxifolia* partially burned log	Ladysmith, B.C.	Collected I. Mounce, July 27, 1938.
8255	*T*	*Pseudotsuga taxifolia* rotted log	Mud Bay, V.I., B.C.	Collected I. Mounce, July 29, 1938.
8256	*T*	Rotted, decorticated log	Westholme, B.C.	Collected I. Mounce, July 26, 1938.
8269	*T*	Burned stump	Oyster River, B.C.	Collected I. Mounce, Aug. 26, 1938.
8271	*T*	*Pseudotsuga taxifolia* fallen log	Oyster River, B.C.	Collected I. Mounce, Aug. 25, 1938.
8277	*T*	*Pseudotsuga taxifolia*	Ladysmith, B.C.	Collected I. Mounce, Aug. 27, 1938.
8279	*T*	*Pseudotsuga taxifolia* railway ties	Deep Bay, B.C.	Collected I. Mounce, July 29, 1938.
8281	*T*	*Pseudotsuga taxifolia*	Deep Bay, B.C.	Collected I. Mounce, July 29, 1938.
8282	*T*	*Pseudotsuga taxifolia*	Saanichton, B.C.	Collected I. Mounce, Aug. 31, 1938.

Each isolate has been subjected to the routine procedure followed in this Laboratory for the identification of fungus cultures (15, p. 529). The culture is grown in the dark at room temperature, on 2% Difco malt agar in 9-cm. Petri dishes, and examined at weekly intervals for six weeks. Observations are made on (i) rate of growth, as measured by radius of colony from the inoculum, which is placed at the edge of the Petri dish, (ii) colour, based on Ridgway's (18) standards, (iii) character of margin, (iv) contour and texture of mat, (v) colour changes in the agar ("Reverse"), and (vi) odour. In addition, the fungus is tested on media containing gallic and tannic acid, the method described by Davidson, Campbell, and Blaisdell (9) being used. This consists in growing the fungi on malt agar to which has been added 0.5% gallic or tannic acid. The formation of a dark brown diffusion zone below and surrounding the inoculum or colony constitutes a positive reaction, and is, in general, characteristic of species producing white rots, whereas failure to produce such a diffusion zone is characteristic of species producing brown rots. The density and extent of the diffusion zones and the inhibitory effect of the acids upon growth of the colonies are constant for each species, and so provide additional diagnostic characters. Microscopic examinations are made at suitable intervals, records being kept of the characteristics of the hyphae, occurrence of secondary spores, special structures, etc.

Data on cultural characters were obtained for each isolate in Table I, and on the basis of this information the cultures were arranged in groups having like characters. By comparison of the cultural characters of the isolates within each group with the cultural characters of named cultures obtained from identified fruit bodies, it was possible to assign the proper specific name to the isolates making up each of the groups. In this way a number of unidentified cultures from rots and fruit bodies were identified, and several isolates received at the Laboratory under misnomers were correctly determined. In all, 12 isolates of *Poria microspora*, 18 isolates of *Trametes serialis*, 1 of *Poria Sequoiae*, 7 of *Polyporus palustris*, and 13 of *Poria carbonica* were studied and from the information so obtained, descriptions of cultures of each of these species prepared, the attempt being made to make the descriptions cover the range of characters encountered in the various isolates. These descriptions are included in a later section.

Interfertility Studies

To corroborate the separations and identifications made on the basis of cultural characters, a number of interfertility tests were made. The method depends on the demonstration by many students of heterothallic Hymenomycetes that every monosporous mycelium obtained from one fruit body of a given species will pair with every monosporous mycelium from another fruit body of the same species from a different source, in such a way as to produce mycelium bearing clamp connections. This complete interfertility is accepted as proof that the fruit bodies belong to the same species. Such interfertility between monosporous mycelia from fruit bodies of different

species has been recorded only twice, by Vandendries (24), and by Routien (20), and in these two interspecific crosses, the mycelium bearing clamp connections was produced sparsely in only a few of the pairings, differing markedly from intraspecific crosses in which every pairing produces mycelium bearing numerous clamp connections. Conversely, complete lack of fertility between the monosporous mycelia from different fruit bodies indicates that the fruit bodies are of different species, although certain exceptions to this have been recorded. In *Coprinus micaceus* (25), *Auricularia auricula-Judae* (1), *Fomes pinicola* (16), and in *Polyporus abietinus* (13), certain groups of isolates compatible among themselves were found to be incompatible with other groups of isolates of the same species. Hence, sterility between monosporous mycelia derived from different fruit bodies is an indication that they are of different species, but not positive proof.

In these investigations four, or in a few crosses only two, monosporous mycelia from each of the isolates to be compared were grown together in pairs in all possible combinations and the resulting mycelia examined for the presence of clamp connections. Formation of clamp connections in all or most of the pairings was accepted as positive proof that the two were of the same species; complete absence of clamp connections in all of the pairings indicated that they belonged to different species.

Cultures of *Poria microspora*, *Trametes serialis*, *Polyporus palustris*, and *Poria Sequoiae* produce fruit bodies in culture, which makes it possible to isolate monosporous mycelia and conduct interfertility tests. Twenty-nine isolates in the four species have been tested in this way, 139 intercollection crosses being made. Fig. 1 shows the results obtained from pairing four monosporous mycelia from each of the isolates Nos. *F*458 and *F*347, both belonging to *Poria microspora*. Every pairing produced mycelium bearing clamp connections, proving that the two isolates are conspecific. This table is typical of all of those obtained when two compatible isolates, i.e. isolates from fruit bodies of the same species, are crossed.

			F347			
			A		a	
			1	4	2	3
F458	A	1	+	+	+	+
		4	+	+	+	+
	a	2	+	+	+	+
		3	+	+	+	+

FIG. 1. *Results obtained by pairing four monosporous mycelia from two fruit bodies of Poria microspora.*

			F6813			
			A		a	
			1	3	2	4
F458	A	1	−	−	−	−
		4	−	−	−	−
	a	2	−	−	−	−
		3	−	−	−	−

FIG. 2. *Results obtained by pairing four monosporous mycelia from Poria microspora fruit body No. F458 with four monosporous mycelia from Trametes serialis fruit body No. F6813.*

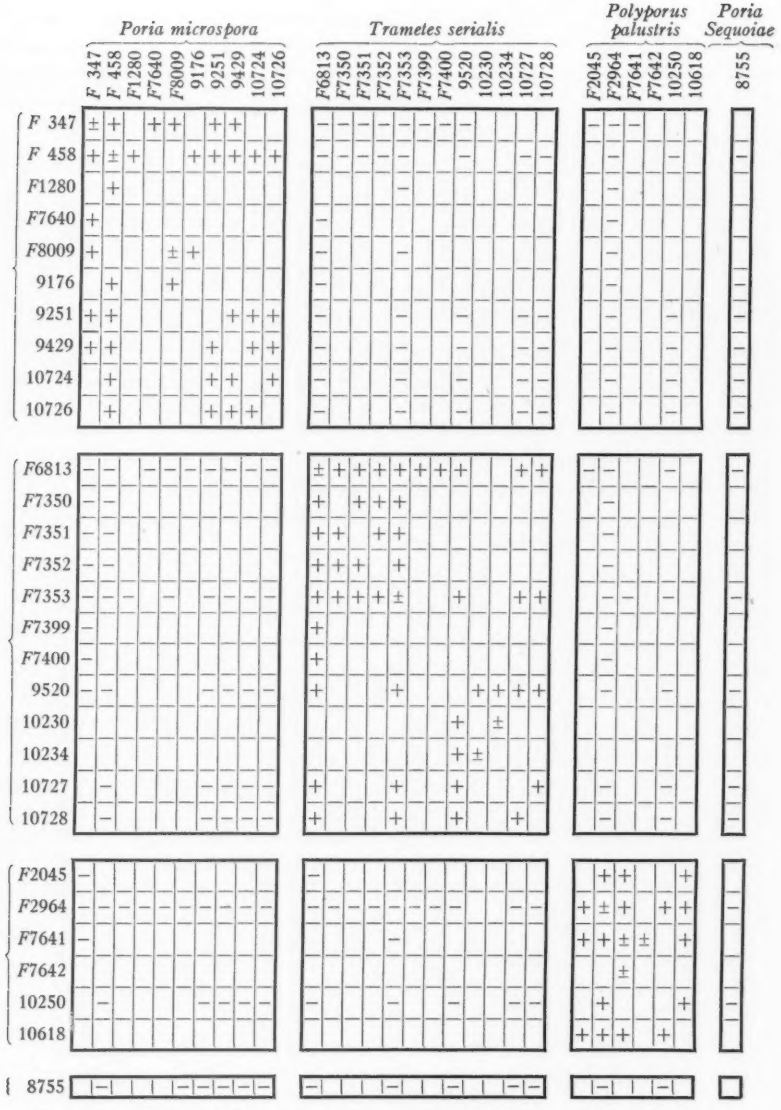
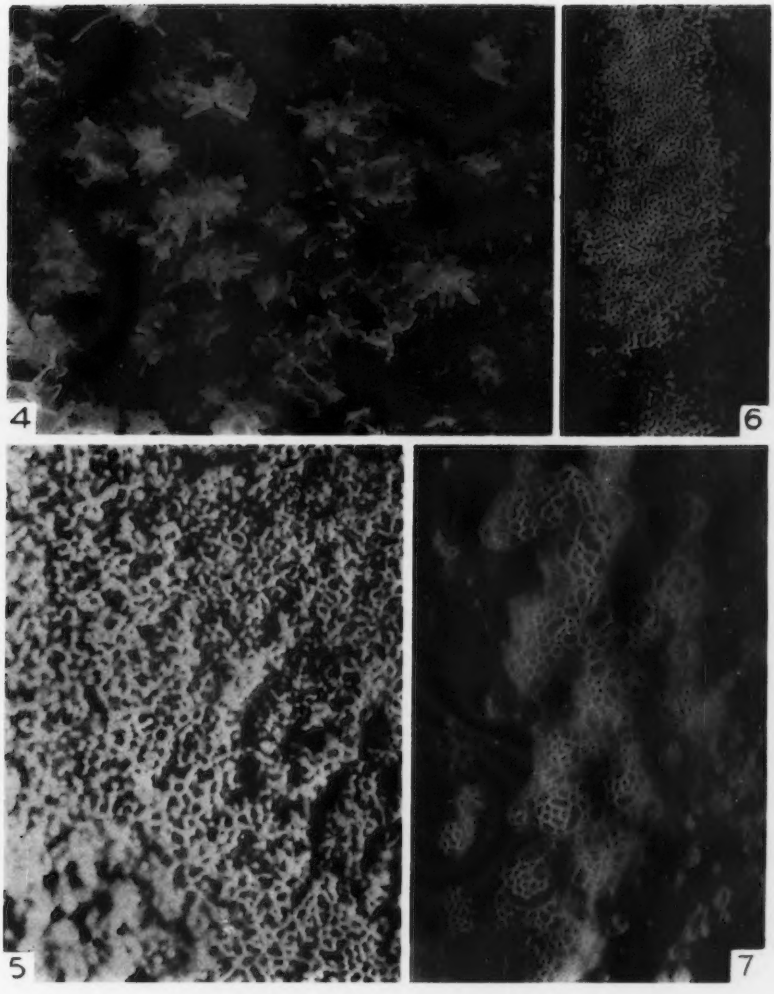
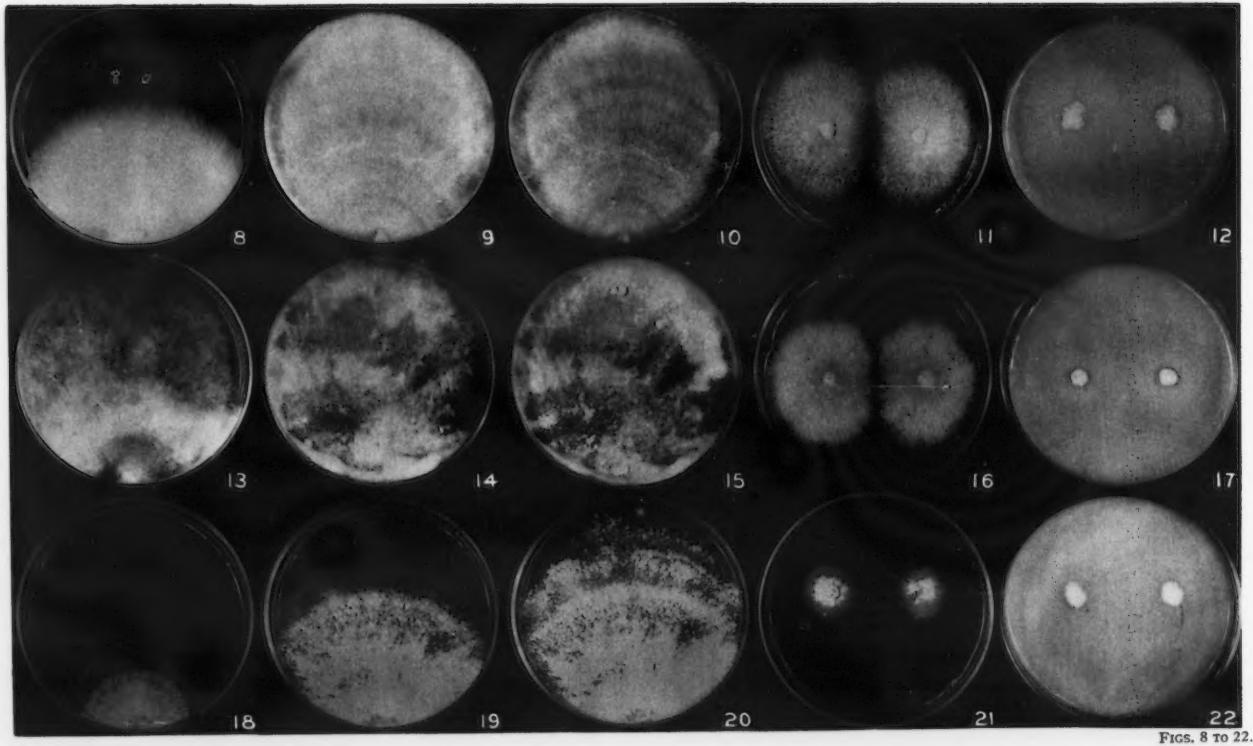


FIG. 3. *Results obtained by pairing, in all possible combinations, series of monosporous mycelia from different fruit bodies of Poria microspora, Trametes serialis, Polyporus palustris, and Poria Sequoiae.*



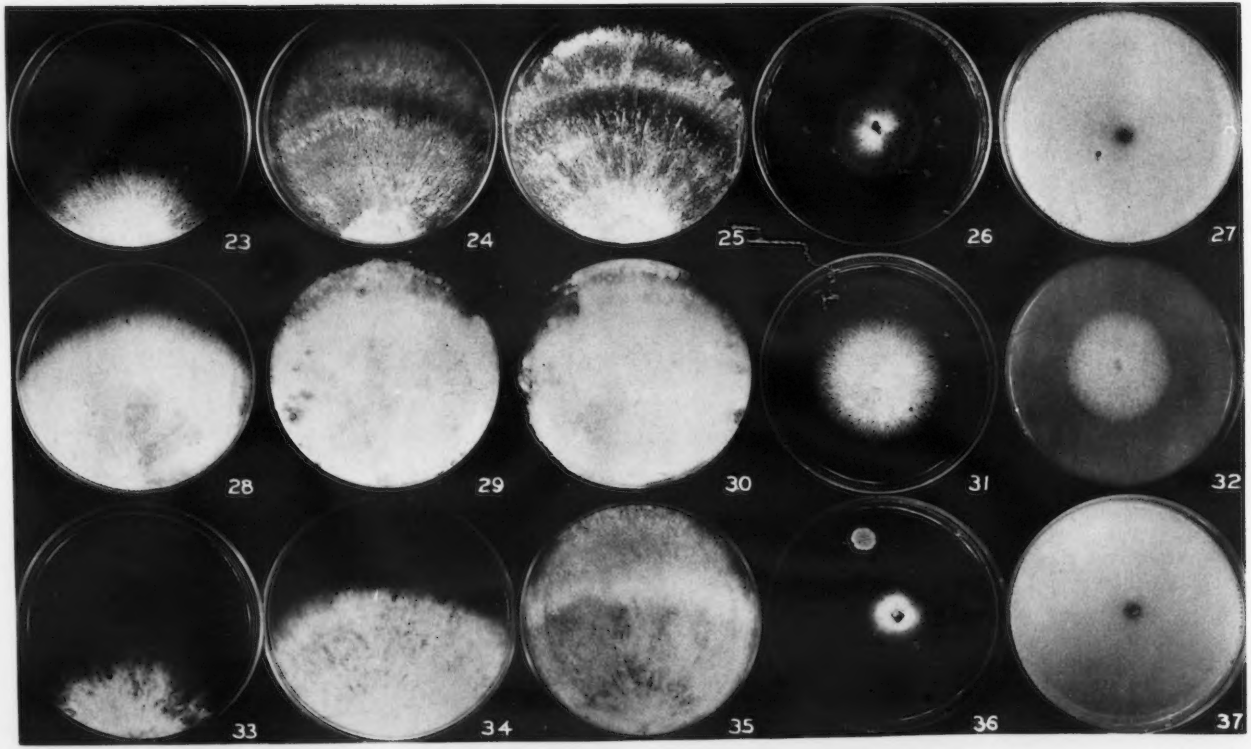
FIGS. 4 TO 7.

Fruit bodies produced in culture. FIG. 4. *Poria microspora*. FIG. 5. *Trametes serialis*. FIG. 6. *Poria Sequoiae*. FIG. 7. *Polyporus palustris*. × 3.5 approximately.



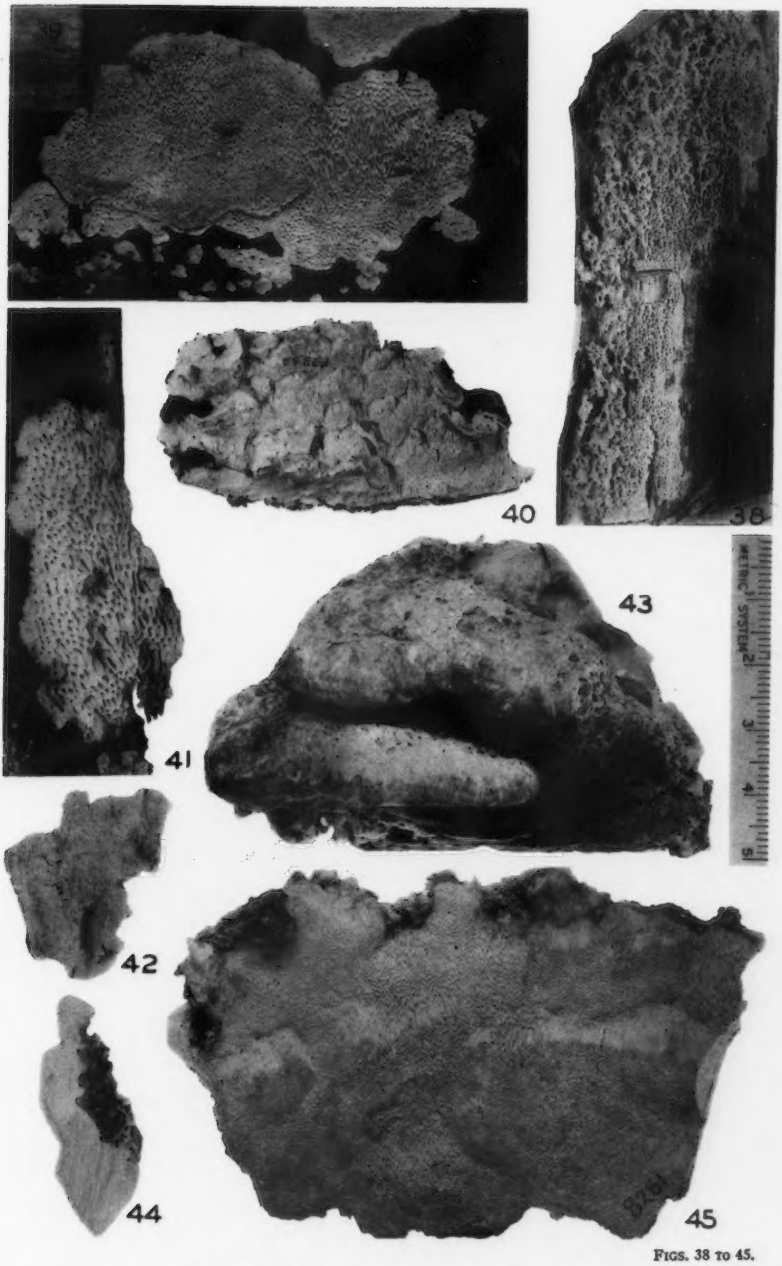
FIGS. 8 TO 22.

Poria microspora No. 9176. FIGS. 8 TO 10: on malt agar, two weeks, four weeks, and six weeks old, respectively. FIG. 11: on gallic acid agar. FIG. 12: on tannic acid agar, one week old. FIGS. 13 TO 17. *Poria microspora* No. 10724. As for *Poria microspora* No. 9176. FIGS. 18 TO 22. *Trametes serialis* No. F7353. As for *Poria microspora*.



FIGS. 23 TO 37.

As for *Poria microspora* (Plate II). FIGS. 23 TO 27. *Poria Sequoiae* No. 8755. FIGS. 28 TO 32. *Polyporus palustris* No. 10250. FIGS. 33 TO 37. *Poria carbonica* No. 8281.



FIGS. 38 TO 45.

Fruit bodies produced in nature. FIG. 38. *Poria microspora.* FIGS. 39 TO 41. *Trametes serialis.* FIG. 42. *Poria Sequoiae.* FIG. 43. *Polyporus palustris.* FIG. 44. *Poria carbonica,* in section. FIG. 45. *Poria carbonica.* ×1 approximately.

Fig. 2 shows a table of pairings between monosporous mycelia of isolates Nos. *F*458 (*Poria microspora*) and *F*6813 (*Trametes serialis*), typical of those obtained when crosses are made between two isolates that are not interfertile. None of the pairings gives rise to mycelium bearing clamp connections, and this complete lack of fertility indicates that the two isolates belong to different species.

The results obtained from all of the intercollection crosses are summarized in Fig. 3, in which a (+) sign is the symbol for results such as those shown in Fig. 1, denoting complete fertility between the two isolates, while a (−) sign stands for complete sterility as illustrated in Fig. 2. The significance of the symbols (±) will be discussed under the individual species. Where no sign appears in a square, no series of pairings was made. It must be mentioned that in certain of the compatible crosses, perfect tables like that shown in Fig. 1 were not obtained, one or two of the 16 pairings failing to produce mycelium bearing clamp connections. It was believed that these failures to pair were attributable to mechanical difficulties such as failure of inocula to grow or attenuation of the mycelia, rather than to genetic differences. Accordingly in Fig. 3, the same sign (+) is used to designate the tables obtained for series of pairings in which all 16 pairings produced mycelia bearing clamp connections, as well as those in which one or two of the pairings failed to react in this way. Without exception, the (−) signs in Fig. 3 denote complete sterility in the crosses, i.e., they all refer to perfect tables like that shown in Fig. 2.

It will be observed that the isolates other than *Poria Sequoiae* 8755 fall into three distinct groups, in each of which the isolates are compatible among themselves, but incompatible with isolates in other groups. This grouping parallels, and so verifies, the grouping made on the basis of cultural characters.

In the key in use in this Laboratory for the identification of fungus cultures the five species included in this study fall close together in that their mats are white or only slightly coloured at the end of six weeks, they all give a negative reaction on gallic and tannic acid agars, and all have nodose-septate hyphae, these being the characters used in the first separations in the key. A brief key for the identification of these five species follows.

1. Mats white or only slightly coloured at the end of six weeks.....2
   Mats coloured.....
2. Diffusion zones present on gallic and tannic acid agars.....
   Diffusion zones lacking.....3
3. Clamp connections present.....4
   Clamp connections lacking.....
4. Fibre hyphae present.....5
   Fibre hyphae lacking.....6

5. Chlamydospores present; colonies 2.0–4.5 cm. diameter on both gallic and tannic acid agars; typically southern.....*Polyporus palustris*
   Chlamydospores lacking; colonies less than 2.0 cm. diameter on gallic and tannic acid agars; widely distributed.....*Trametes serialis*
6. Characteristic thick-walled, much-branched hyphae present; abundant conidia and chlamydospores.....*Poria carbonica*
   No such special hyphae; no conidia, but chlamydospores may be present.....7
7. Chlamydospores present; occurring on variety of coniferous woods, especially on timber in service.....*Poria microspora*
   Chlamydospores lacking; limited to *Sequoia sempervirens*.....*Poria Sequoiae*

Species Studied

1. *Poria microspora* Overholts, n. sp.

Cultural Characters

From Table I it may be seen that 12 isolates of this species have been studied, of which 11 were obtained from rots and only one from a sporophore. Irrespective of their source, however, the cultures are remarkably uniform and readily recognizable. A description of the cultures, based on the 12 isolates listed in Table I, follows.

Growth moderately rapid, plates covered in (2–) 3 weeks (Figs. 8–10, 13–15). Advancing zone even, appressed and hyaline in some isolates, or with raised aerial mycelium extending to limit of growth. Mat white or with tinge of pale cartridge buff to cinnamon buff at edges, slightly raised, loosely arranged, cottony to woolly, frequently grown against sides of Petri dish and across lid, forming scattered fruit bodies after two to three weeks in some isolates, later in others, these consisting of waxy granules or plates coalescing to form foliose or, more rarely, irregularly pored fruiting surfaces, which produce copious spore deposits (Fig. 4). Reverse unchanged. Odour none. On gallic and tannic acid agars, no diffusion zones, diameter 3.0–5.6 cm. on gallic acid agar in one week, no growth or only a trace on tannic acid agar (Figs. 11, 12, 16, 17) (Group 2).

FIGS. 46 TO 53. *Poria microspora.* FIG. 46. *Hyphae from advancing zone.* FIG. 47. *Thick-walled hyphae from aerial mycelium.* FIG. 48. *Chlamydospores.* FIG. 49. *Hypha from submerged mycelium.* FIG. 50. *Basidia produced in culture.* FIGS. 51 TO 53. *Hypha, basidia, and basidiospores from fruit body produced in nature.*

FIGS. 54 TO 59. *Trametes serialis.* FIG. 54. *Hyphae from advancing zone.* FIG. 55. *Hyphae from aerial mycelium.* FIG. 56. *Fibre hyphae.* FIGS. 57, 58. *Basidia and basidiospores produced in culture.* FIG. 59. *Basidia from fruit body produced in nature.*

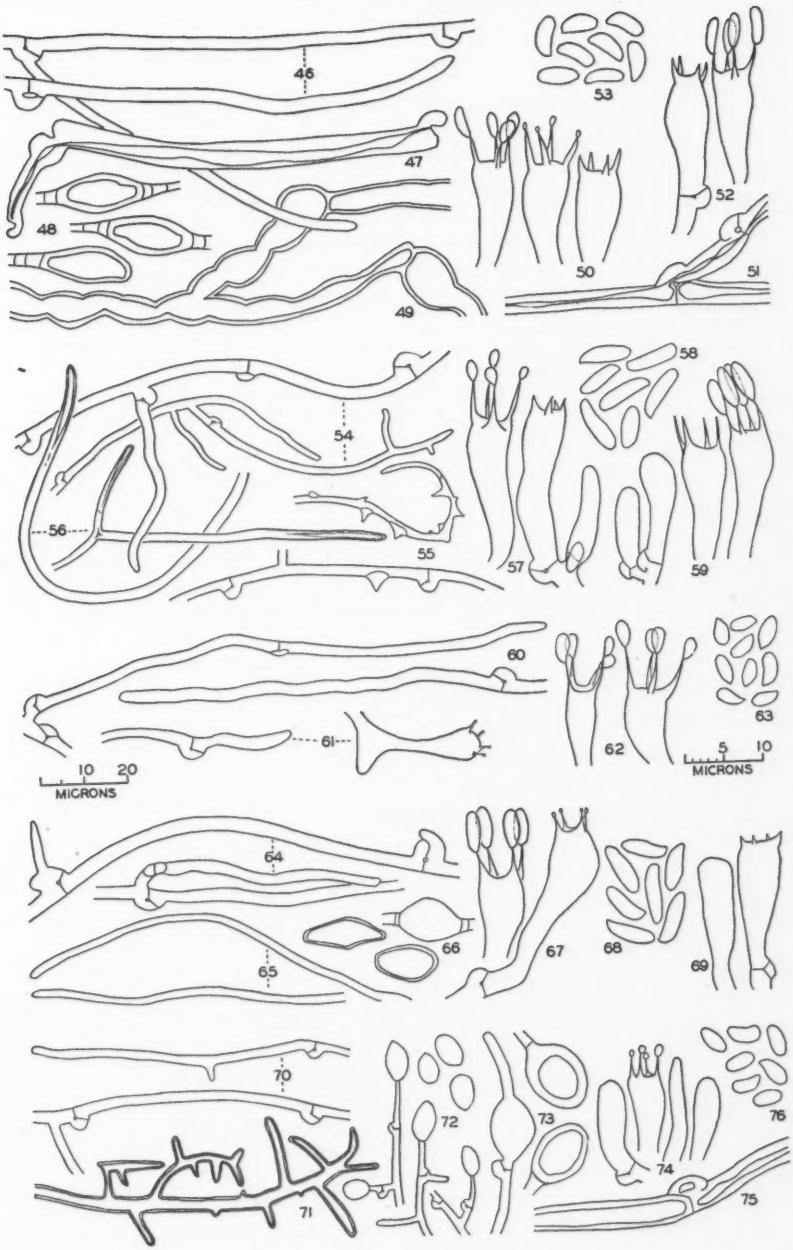
FIGS. 60 TO 63. *Poria Sequoiae.* FIG. 60. *Hyphae from advancing zone.* FIG. 61. *Immature basidia produced in culture.* FIGS. 62, 63. *Basidia and basidiospores from fruit body produced in nature.*

FIGS. 64 TO 69. *Polyporus palustris.* FIG. 64. *Hyphae from advancing zone.* FIG. 65. *Fibre hyphae.* FIG. 66. *Chlamydospores.* FIGS. 67, 68. *Basidia and basidiospores produced in culture.* FIG. 69. *Basidia from fruit body produced in nature.*

FIGS. 70 TO 76. *Poria carbonica.* FIG. 70. *Hyphae from advancing zone.* FIG. 71. *Hypha from aerial mycelium.* FIG. 72. *Conidiophores and conidia.* FIG. 73. *Chlamydospores.* FIGS. 74 TO 76. *Basidia, hypha, and basidiospores from fruit body produced in nature.*

Figs. 46 to 49, 54 to 56, 60 to 61, 64 to 66, 70 to 73. ×700.

Figs. 50 to 53, 57 to 59, 62 to 63, 67 to 69, 74 to 76. ×1250.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MICRONS
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Advancing zone: hyphae hyaline, thin-walled, contents staining in phloxine, nodose-septate, branched at and between septa, 2.2–6.0 μ diameter (Fig. 46). *Aerial mycelium:* (*a*) nodose-septate hyphae as in advancing zone, in some isolates occasionally up to 9.0–15.0 μ diameter, with walls irregularly thickened and lumina relatively narrow (Fig. 47); (*b*) chlamydospores rare to numerous, with walls slightly thickened, contents staining deeply in phloxine, intercalary or terminal, 8.0–19.5 × 6.0–13.5 μ (Fig. 48). *Fruit body:* (*a*) nodose-septate hyphae as in advancing zone; (*b*) basidia 13.0–18.0 × 5.4–6.3 μ, bearing four sterigmata and spores (Fig. 50); (*c*) basidiospores hyaline, even, oblong-ellipsoid, apiculate, somewhat flattened on one side, 4.5–6.3 × 2.2–2.7 μ (Fig. 53). *Submerged mycelium:* (*a*) hyphae as in aerial mycelium; (*b*) hyphae with thick walls and irregular swellings up to 16.5 μ diameter, found only in older cultures (Fig. 49); (*c*) crystals numerous, octahedral.

The faintly pinkish tinge of the mycelium, frequently too pale to be identified in Ridgway's (18) scale, and the early formation of characteristic foliose fruit bodies with heavy spore deposits, serve to differentiate this fungus from other species.

Interfertility Studies

This differentiation has been completely verified by interfertility tests. Each of 10 isolates of *Poria microspora* was crossed with one or more other isolates of the same species and with representative cultures of *Trametes serialis*, *Polyporus palustris*, and *Poria Sequoiae*, the results being presented in Fig. 3. It will be observed that the isolates of this species are completely compatible with other isolates of the same species and completely incompatible with isolates of the other species. This, in conjunction with the evidence of cultural and morphological studies, provides adequate proof that this constitutes a species distinct from *Trametes serialis*, and from the other species listed.

Twelve monosporous mycelia from isolate No. *F*458 were grown together in pairs in all possible combinations, and the resulting mycelia examined for the presence of clamp connections. Fig. 77 shows that the mycelia fall into

		A					a					
		1	4	5	10	11	2	3	6	7	8	9	12
A	1		−	−	−	−	+	+	+	+	+	+	+
	4	−		−	−	−	+	+	+	+	+	+	+
	5	−	−		−	−	+	+	+	+	+	+	+
	10	−	−	−		−	+	+	+	+	+	+	+
	11	−	−	−	−		+	+	+	+	+	+	+
a	2	+	+	+	+	+		−	−	−	−	−	−
	3	+	+	+	+	+	−		−	−	−	−	−
	6	+	+	+	+	+	−	−		−	−	−	−
	7	+	+	+	+	+	−	−	−		−	−	−
	8	+	+	+	+	+	−	−	−	−		−	−
	9	+	+	+	+	+	−	−	−	−	−		−
	12	+	+	+	+	+	−	−	−	−	−	−	

FIG. 77. *Results obtained by pairing in all possible combinations, 12 monosporous mycelia from Poria microspora fruit body No. F458.*

two groups, the members of each group remaining sterile when paired with each other, but being interfertile with members of the other group. Similar results were obtained from pairings of monosporous mycelia from isolates Nos. *F*347 and *F*8009. Hence *Poria microspora* exhibits the bipolar type of interfertility.

History and Nomenclature

A brown, cubical rot was encountered in Sitka spruce of aeroplane quality during and after the war of 1914–1918, and investigations were undertaken in Canada and Great Britain. Working independently, Dr. Irene Mounce in Canada and Cartwright (6) in England, isolated a fungus that appeared to both investigators to be responsible for much of this rot. Cartwright's determination of the culture as *Trametes serialis* was based on the similarity between his isolates and the description of *T. serialis* presented by Snell (22), and the subsequent comparison of his cultures with cultures of *T. serialis* obtained from the Forest Products Laboratory at Madison, Wis. From Snell's (22, p. 15) descriptions and illustrations of fruit bodies of *T. serialis* produced on decayed timbers in the fungus pit at Madison and of the cultures obtained therefrom, it would appear that this fungus was not *T. serialis*, but was identical with *Poria microspora*. On the other hand, Snell's Plate 11, Fig. 1, shows fruit bodies that are typical for *Trametes serialis*, suggesting that he was dealing with both *T. serialis* and *Poria microspora*. This is substantiated by Snell's (23) statement on the difficulty of making "exact statements as to the occurrence and identity of *T. serialis*" because "the limits of the species are variously interpreted by mycologists" and because "within mills there are so many abnormal fungus growths". In the same publication (23) he noted that he had "found spores from fungi grown under mill conditions are occasionally smaller than the measurements usually given for a species". This would also suggest that at least some of his specimens of "*T. serialis*" were actually *Poria microspora* which has spores 4.5–6.5 × 2.5–3.0 μ as opposed to spores 7.0–9.0 × 2.0–3.0 μ for *T. serialis*. This would imply that the description of *T. serialis* by Snell on which Cartwright based the determination of his unknown culture may have been actually a description of the same fungus, erroneously named.

To verify his determination Cartwright obtained one or more cultures of *Trametes serialis* from Dr. C. Audrey Richards of the Forest Products Laboratory at Madison, and found them to be identical with his culture. Unfortunately these also appear to have been wrongly identified. Dr. Richards has kindly sent to this Laboratory the three cultures in their stock culture collection under the name *T. serialis*. Cultural and interfertility studies have shown that two of these cultures, Nos. *F*7641 and *F*7642 in Table I are *Polyporus palustris*, and that the third, No. *F*7640 in Table I, is *Poria microspora*. Concerning this last, Dr. Richards has written "No. 575 was isolated by Dr. Humphrey from the wood of Douglas fir in Oregon, infected with *Trametes serialis*". This culture is identical with cultures of *Poria microspora*, and as will be seen from Fig. 3, is interfertile with No. *F*347, and sterile with

Trametes serialis No. *F*6813. It is therefore a culture of *Poria microspora* and may well have been the culture obtained by Cartwright[1] for comparison with his unknown. It should be noted that this culture was obtained from a rot and must have been determined on the basis of rot characters.

Connection of the cultures of this fungus with a fruit body could be accomplished only by comparison of cultures. Accordingly they were compared with cultures of some 150 species in the stock culture collection at the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, but were found to differ from all of these. Since the rot is prevalent in worked lumber of British Columbia species, Dr. Irene Mounce and Dr. J. E. Bier made many collections and cultures from fruit bodies on various coniferous hosts in several districts of that province. This was necessarily haphazard, since there was no means of knowing what kind of fruit body to look for. Finally in May, 1942, Dr. Bier collected a fruit body on *Picea sitchensis* in the Queen Charlotte Islands, B.C., cultures of which proved to be identical with those obtained from rot. The culture from this fruit body, No. 10724, is interfertile with Nos. *F*458, 9251, 9429, and 10726, and intersterile with cultures of *Trametes serialis* (Fig. 3). This provides conclusive evidence that the fruit body is that of the rot-producing organism, and that it is different from *Trametes serialis*. It was submitted to Dr. L. O. Overholts, who agreed that it appeared to be an undescribed species and who has prepared the following description.

Poria microspora Overholts, n. sp. (Figs. 38, 51–53)

Fructificatio annua, effusa, inseparabilis, evanescens, 2–3 mm. crassa, margine tenui, primo sterili demum fertili, coriacea in humido, fragilis in sicco, albida vel straminea vel cinerea; tubulis 1–2.5 mm. longis, 2–3 pro mm., angulosis, dissepimentibus primo tenuis, integrisque, dein interdum laceratis confluentibusque; subiculo distincto, tenuissimo, albo, hyphis variabilibus, simplicibus vel ramosissimis, 3–4.5 μ diam., nodoso-septatis, muris valde crassis, sporis breve-cylindricis vel anguste-ellipsoideis, levibus, hyalinis, unolatere complanatis, 4.5–6.5 $\times$ 2.5–3.0 μ; basidiis tetrasporis, clavatis, 13–18 $\times$ 5–6 μ.

Hab.: in ligno emortuo Coniferarum.

"Effused for several centimetres, annual and apparently likely to disintegrate and largely disappear in a short time, 2–3 mm. thick, the margin thinning out to a sterile membrane that eventually becomes fertile, the sterile margin entirely disappearing, not separating from the substratum without taking fragments of the rotten wood with it, coriaceous when fresh, drying rather brittle, white or straw-colour to cinereous; tubes 1–2.5 mm. long, the mouths 2–3 per mm., angular, rather thin-walled and entire or becoming torn and confluent and as much as 1 mm. diameter; subiculum distinct, papery-thin, white; spores short-cylindric or somewhat narrow-ellipsoid, flattened on one side, smooth, hyaline, 4.5–6.5 $\times$ 2.5–3.0 μ; basidia four-spored, clavate, 13–18 $\times$ 5–6 μ; hyphae of subiculum variable, some simple, others much

[1] *In a letter dated May 11, 1942, Mr. Cartwright wrote "When you publish an account of your work, I should be glad if you would add a short note in order to make it clear that I am in agreement, and that the work carried out by us here under the name of T. serialis is the Madison T. serialis, being identified from a culture received from them. This I think would help to avoid future trouble, if further physiological or other work was carried out elsewhere."*

branched, 3–4.5 μ diameter, the walls greatly thickened, cross walls and clamp connections rather abundant.

"On wood of coniferous trees. Type collected on *Picea sitchensis*, Queen Charlotte Islands, B.C., Canada, May 15, 1942, by J. E. Bier (Dept. of Agr., Ottawa, Mycol. Herb. No. 10724; Overholts Herb. No. 23826). Two other sporophores of what seems to be the same fungus are in Overholts Herbarium, one collected from the floor of a building at Sharon, Wis., October 5, 1929, and forwarded by C. A. Richards under the Forest Products Laboratory (U.S.) No. 39270. The other specimen was collected by Spaulding and Hansbrough, at Mystic, Conn., on a southern pine beam in a whaling boat 100 years old, March 18, 1942. This was sent to R. W. Davidson, who forwarded it to me under United States Division of Forest Pathology No. 94153."

The evanescent nature of the fruit body appears to be characteristic. Snell (22), in describing fruit bodies of "*Trametes serialis*" produced on decayed mill timbers, recorded that "the spent fruit bodies of *Trametes serialis* left in the fungus pit soon disappeared". This brief existence may account for the fact that the fungus has not been collected more frequently.

Hosts and Distribution

This fungus, *Poria microspora*, has been isolated from rots in the following hosts:

Picea sitchensis (Bong.) Carr.: isolated by Cartwright (6) from timber imported into Great Britain from Canada, by Mounce (unpublished records) from standing trees and from timber in mills in British Columbia, by Bier from rot in standing trees in Queen Charlotte Islands, B.C., and from a fruit body produced on a standing tree in this same locality.

Pseudotsuga taxifolia (Poir.) Britt.: isolated by Cartwright and Findlay (7) from timber imported into Great Britain from Canada, by Humphrey from decayed wood in Oregon, U.S.A. (Madison culture No. 575), and by Dr. C. W. Fritz from lumber in use in a building in Toronto, Ont.

Tsuga heterophylla (Raf.) Sarg.: isolated by the author from rot in a standing tree in Vancouver, B.C.

Pinus Banksiana Lamb.: isolated by Fritz and Atwell (10) from railway ties.

In addition, Dr. C. W. Fritz, Forest Products Laboratories, Ottawa, in making isolations from spore traps exposed in a lumber yard at Calumet, P.Q., in 1938, obtained two single spore cultures which, on pairing, formed a mycelium bearing clamp connections. This proved to be *Poria microspora*. Hence the fungus fruited in this locality in Eastern Canada at that time, presumably on lumber cut in the vicinity. No fruit bodies were collected then or in 1941 when the author visited the same lumber yard, so that no statement concerning the host can be made.

These scattered records convey no adequate picture of the host range or geographic distribution. The apparent rarity of fruit bodies in nature has necessitated dependence on isolations of the fungus from wood in various

stages of decay for proof of its presence, and the difficulty of identifying the cultures so obtained has made publication of records impossible. It is true that certain records under the name *Trametes serialis* refer to this fungus (*Poria microspora*) but these can be recognized in only a few instances. It would appear, however, that *Poria microspora* may be found across Canada, and may occur on a number of species of coniferous trees.

Decay

The decay produced by this organism in Sitka spruce and Douglas fir has been well described by Cartwright (6), Cartwright *et al.* (8), and Cartwright and Findlay (7), under the name *Trametes serialis*. In describing the rot, Cartwright and Findlay (7) write "The first indications of incipient decay ("dote") are faint streaks or elongated patches of yellowish-brown or pinkish-brown discoloration, and if these are tested with the tip of a penknife blade, it will be found that the fibres break off short and do not come up in a splinter, thereby indicating brittleness of the wood. It has been found by Cartwright *et al.* (1931) that even slight incipient decay by this fungus causes an immediate and serious loss of strength, particularly in toughness (i.e. resistance to suddenly applied stresses).

"In Douglas fir (Oregon pine) incipient decay may appear in unseasoned timber as small irregular dark areas over the surface, rather suggesting contact with drops of oil; as these become dry they show minute cracks and the wood in these areas crumbles if tested.

"In a more advanced stage pockets of brown cuboidal rot are formed, and eventually the whole piece of wood may break up into cubical pieces which are darker in colour than the normal and crumble readily if crushed."

After reading this paper in manuscript Dr. C. W. Fritz generously contributed her observations on the similarity between the incipient stages of the decays caused by *Poria microspora* and *Fomes Pini*. She wrote (Letter April 7, 1943), "In the incipient stage the wood shows marked purplish discoloration. This has come to my attention in two lots of material submitted for examination. In the first instance I thought the discoloration was probably caused by *Fomes pini*, but all the cultures made yielded the fungus described in your paper as *Poria microspora*. Last autumn Mr. Findlay sent me some small pieces of Douglas fir showing the same discoloration with a request to know whether I would consider the stain due to attack by *Fomes pini*. I immediately thought of the material examined previously and made cultures. They also proved to be *Poria microspora*. It seems probable that much of the incipient decay in Douglas fir, which has previously been thought due to *Fomes pini*, is really the result of attack by *Poria microspora*."

2. *Trametes serialis* Fries

Cultural Characters

Eighteen isolates of *Trametes serialis*, 16 of which were obtained from fruit bodies or decay associated with fruit bodies, have been studied, and have provided the information for the following description.

Growth slow, plates covered in five to six weeks (Figs. 18–20). Zone of newest growth 1–2 cm. broad, appressed, hyaline, changing abruptly to white mat, appressed, felty, soon pitted and corrugated to form irregularly pored surface (one to three weeks) which finally extends over whole surface of colony (Fig. 5). Reverse unchanged. Odour none or faintly fruity. On gallic and tannic acid agars, no diffusion zones (may be slight browning around colony on gallic acid agar), diameter 1.0–2.0 cm. on gallic acid agar, trace to 1.0 cm. on tannic acid agar (Figs. 21–22) (Group 2).

Advancing zone: hyphae hyaline, thin-walled, contents staining in phloxine, nodose-septate, 1.5–4.5(–6.0) μ diameter (Fig. 54). *Aerial mycelium:* (*a*) hyphae as in advancing zone, frequently with numerous small pointed projections in which walls are thickened and refractive (Fig. 55); (*b*) fibre hyphae very numerous, walls thick and refractive, apparently aseptate, occasionally branched, 1.5–3.0 μ diameter (Fig. 56). *Fruit body:* (*a*) thin-walled and (*b*) fibre hyphae as in aerial mycelium; (*c*) basidia 19.8–22.5 $\times$ 4.5–6.3 μ, bearing four sterigmata up to 4.5–7.2 μ long (Fig. 57); (*d*) basidiospores hyaline, even, cylindric, (5.4–) 6.3–8.1 $\times$ 2.2–2.7 μ (Fig. 58). *Submerged mycelium:* (*a*) nodose-septate hyphae as described above; (*b*) crystals numerous, octahedral or plate-like.

The fungus in culture is readily recognizable, by reason of the characteristic fruiting surface which appears early and eventually spreads over the entire colony, the abundance of fibre hyphae, and the nodose-septate hyphae with small refractive projections along their walls.

Interfertility Studies

Twelve isolates of *Trametes serialis* have been paired among themselves and with representative isolates of *Poria microspora*, *Polyporus palustris*, and *Poria Sequoiae*. From Fig. 3 it may be observed that there is complete interfertility among the isolates of *T. serialis*, this confirming the identifications based on examinations of fruit bodies and cultures derived therefrom, and complete lack of interfertility when isolates of this species are crossed with isolates of the other species. Hence, *Trametes serialis* is distinct from *Poria microspora*, *Polyporus palustris*, and *Poria Sequoiae*, each of which has been confused with it.

Twelve monosporous mycelia from isolate No. *F*6813 were grown together in pairs in all possible combinations and the resulting mycelia examined for the presence of clamp connections. The results presented in Fig. 78 show that the mycelia fall into two groups, and this was again demonstrated when a similar series of pairings between 12 monosporous mycelia from isolate No. *F*7353 was made. These serve to corroborate the findings of Robak (19) who showed that *Trametes serialis* exhibited the bipolar type of interfertility.

It will be observed from Fig. 3 that when isolates Nos. 10230 and 10234 were crossed, a bipolar table, symbolized by ($\pm$), was obtained, showing that the two isolates were in fact from the same individual plant. The explanation is that the two fruit bodies from which the isolates were obtained were collected by two collectors working over a small area in a mill yard, and that both made collections from the same plant. Proof of this was possible, however, only be means of interfertility studies.

History and Nomenclature

European literature on *Trametes serialis* indicates that it is a well-defined species, occurring on trunks of coniferous trees, particularly in mountainous regions (4). It has also been shown to be the cause of decay in timber in houses by Mez (14), and Bondartzeff (3), in mines by Pilât (17), and in rail-

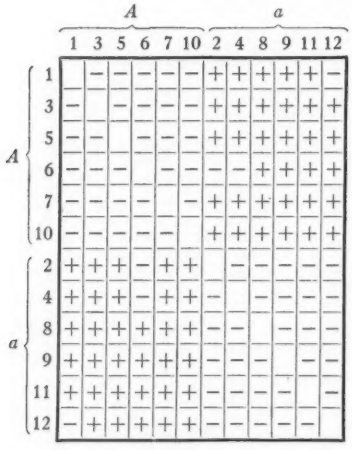

FIG. 78. *Results obtained by pairing, in all possible combinations, 12 monosporous mycelia from Trametes serialis fruit body No. F6813.*

way ties by Walek-Czernecka (26). In America, records of its occurrence based on fruit bodies are probably authentic, since taxonomists, such as Lowe (12), and Shope (21), have provided keys and descriptions that allow for the identification of sporophores. On the other hand, records based on rots or cultures obtained from rots must be accepted with reservation, since it appears that many such identifications have been in error. Thus (Table I) Dr. W. H. Snell's culture 19*M* (10250), which is presumably the one used by Baxter (2) as the basis for his description of the cultural characters of *T. serialis*, is in fact *Polyporus palustris*, as will be shown below. Of the three isolates received from the Forest Products Laboratory at Madison, Wis., one (*F*7640) proved to be *Poria microspora*, the others (*F*7641 and *F*7642), *Polyporus palustris*. In addition, the records provided by Cartwright and his associates, frequently referred to in textbooks e.g. Boyce (5) and elsewhere, must now be transferred to *Poria microspora*. Rigid delimitation of the species, especially as it occurs in culture, will be necessary in the future to avoid perpetuation of these errors.

The fruit body of this species has been adequately described by Lowe (12), Shope (21), and others. Mention should be made, however, of the variability in pore size encountered in different collections of this fungus. Pores ranged from one per mm. (Fig. 41) to two to three per mm. (Fig. 40) one collection showing coarse and fine pores side by side (Fig. 39). These collections showed identical microscopic characters, yielded similar cultures, and were interfertile, so that there is no doubt that they belong to the same species.

Hosts and Distribution

Records, based on fruit bodies and cultures, have been established in this Laboratory for the occurrence of *T. serialis* on *Picea mariana* (Mill.) B. S. P.

and *P. glauca* (Moench) Voss in Quebec, on *P. sitchensis* (Bong.) Carr. in British Columbia, on *Pinus Strobus* L. in Quebec, on *Prunus serotina* Ehrh., *Acer rubrum* L., and *Tsuga canadensis* (L.) Carr. in Pennsylvania, on a coniferous log used as a bridge support in Quebec, and on a coniferous log in use in a gold mine, 3600 ft. below ground, in Northern Ontario. These records are scattered but serve to confirm Shope's (21) statement that the species occurs on "various coniferous and deciduous hosts", and is "widespread in the United States", and presumably in Canada, also.

3. *Poria Sequoiae* Bonar

Poria Sequoiae is known to occur only on *Sequoia sempervirens* (Lamb.) Endl., and is, therefore, restricted in its geographic range to the relatively small area in which its host grows. It is included in this series because at one time it was suspected that it might be identical with *Poria microspora*, the spore sizes of the two species being comparable, and because it has been suggested by Baxter (2) that *Poria Sequoiae* is synonymous with *Trametes serialis*.

Dr. Lee Bonar kindly provided a culture of this species, the description of which follows:

Growth moderately rapid to slow, plates covered in four to five weeks (Figs. 23–25). Advancing zone even, appressed and hyaline in zone 1 cm. wide. Mat white, appressed, subfelty to farinaceous, after three to four weeks becoming thickened along radial lines, slightly raised, waxy, with irregularly pored surface over large areas, producing heavy spore deposits (Fig. 6). Reverse unchanged. Odour none. On gallic and tannic acid agars no diffusion zones, diameter 2.0 cm. on gallic acid agar, trace of growth on tannic acid agar (Figs. 26, 27) (Group 3).

Advancing zone: hyphae hyaline, nodose-septate, 1.5–4.5 μ diameter (Fig. 60). *Aerial mycelium:* hyphae as in advancing zone. *Submerged mycelium:* (*a*) hyphae as in advancing zone; (*b*) crystals large, octahedral.

The culture resembles that of *Trametes serialis* in growth rate, but is readily distinguished from it by the absence of fibre hyphae and the resultant lack of toughness in the mat.

Interfertility Studies

From Fig. 3 it may be seen that monosporous mycelia of *Poria Sequoiae* have been paired with those of *Poria microspora*, *Trametes serialis*, and *Polyporus palustris*, and that complete lack of interfertility has been found in all the crosses. This suggests very strongly that *Poria Sequoiae* is distinct from each of these species. If *Poria Sequoiae* and *Trametes serialis* were conspecific as Baxter (2) suggests, it would be expected that some of the crosses, at least, would show interfertility.

A fruit body of *Poria Sequoiae* (Fig. 42) was received from Dr. Bonar, and has been compared with fruit bodies of *Trametes serialis*. The fragile nature of the fruit body, and the readiness with which it is "attacked by such mold-producing fungi as species of *Penicillium*" (11), the small pores "averaging 4 to 5 per mm.", the absence of fibre hyphae in the trama, and the ellipsoidal spores "smooth, hyaline, apiculate, 4.5 to 6 $\times$ 2 to 3.5 microns" (Figs. 62, 63), are all characters that separate this species from *Trametes serialis*. Hence, from these studies of cultures, interfertility reactions, and

the morphology of the fruit body, it would appear that *Poria Sequoiae* Bonar should retain specific rank.

4. *Polyporus palustris* Berk. & Curt.

This species is southern in its range and has not been reported from Canada. It has been included here only because certain cultures received under the name *T. serialis* proved to be *Polyporus palustris* and the criteria used in the separation of these species may prove useful to other investigators. The following description of the cultural characters of this species is based on a study of the six isolates listed in Table I.

Growth moderately rapid, plates covered in three to four weeks (Figs. 28–30). Advancing zone even, downy white mycelium extending to limit of growth. Mat white, slightly raised, downy to cottony-floccose, becoming thin felty in the older parts of some isolates, after two to three weeks with scattered small raised balls of compact mycelium, these gradually increasing in size and frequently coalescing, with surface at first floccose, then compact and covered with more or less regular pores (Fig. 7). Reverse unchanged. Odour not noteworthy. On gallic and tannic acid agars, no diffusion zones, diameters of colonies 2.0–4.5 cm. on both media (Figs. 31, 32) (Group 1).

Advancing zone: hyphae hyaline, nodose-septate, 2.2–4.5 μ diameter (Fig. 64). *Aerial mycelium:* (*a*) hyphae as in advancing zone, rare in older cultures; (*b*) fibre hyphae, with walls thick and refractive, lumina narrow or apparently lacking, aseptate, rarely branched, 1.5–3.0 μ diameter, curving and interwoven (Fig. 65); (*c*) chlamydospores rare, intercalary or terminal, with thin walls, 10.5–19.5 $\times$ 7.5 –12.0 μ (Fig. 66). *Fruit body:* (*a*) thin-walled and (*b*) fibre hyphae as in aerial mycelium; (*c*) basidia about 6.0 μ diameter bearing four spores (Fig. 67); (*d*) basidiospores hyaline, even, cylindric, flattened on one side, apiculate, 6.0–9.0 $\times$ 2.0–3.0 μ (Fig. 68). *Submerged mycelium:* (*a*) hyphae as in advancing zone; (*b*) chlamydospores as in aerial mycelium, fairly numerous; (*c*) broad hyphae, with walls slightly thickened, nodose-septate, but with clamp connections frequently distorted, contents staining deeply in phloxine, 7.5–9.0(–15.0) μ diameter, seen only in older cultures.

The white, cottony mycelium, with isolated patches of raised, well-formed fruiting surfaces, and the abundance of fibre hyphae, along with the rapid growth on gallic and tannic acid agars, are cultural characters that aid in the separation of this from other species.

Interfertility Studies

In Fig. 3 are presented the results obtained when isolates of *P. palustris* were paired among themselves and with representative isolates of the other species. These show that the isolates of *P. palustris* are interfertile among themselves but incompatible with other species. From Table I it may be seen that of the isolates of this species used in the interfertility tests summarized in Fig. 3, only Nos. *F*2045 and 10618 were received under the name *P. palustris*, the remainder having been labelled *T. serialis*. Interfertility tests have allowed for positive identification of these cultures.

Twelve monosporous cultures of isolate No. *F*2964 were grown together in pairs in all possible combinations and the resulting mycelia examined for the presence of clamp connections. Fig. 79 shows that this fungus exhibits the bipolar type of interfertility. This was confirmed by a second series of pairings among the 10 monosporous mycelia of isolate No. *F*7641.

When four mycelia from isolate No. *F*7641 were paired with four mycelia from isolate No. *F*7642, a bipolar table was obtained. Hence Nos. *F*7641 and *F*7642 are isolations of the same individual. According to Dr. Richards from whom the two cultures were received "No. 662 (= *F*7642) was isolated

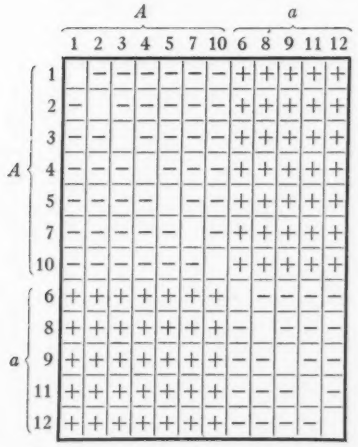

FIG. 79. *Results obtained by pairing, in all possible combinations, 12 monosporous mycelia from Polyporus palustris fruit body No. F2964.*

from a spore from an abortive sporophore which had been raised in culture". On this evidence, the sporophore must have been on Madison culture 661 (= *F*7641).

Since the cultures of *P. palustris* erroneously determined as *T. serialis* were all isolated from sporophores, it appears that the difficulty lies in the identification of sporophores of these species. The cultural and interfertility studies only serve to show the necessity for more reliable criteria for the identification of the fruit bodies. The fruit body from which culture No. *F*2045 was isolated, is illustrated in Fig. 43.

5. *Poria carbonica* Overholts, n. sp.

A study of 13 isolates of *Poria carbonica* has made possible the preparation of the following description of its cultural characters.

Growth moderately rapid to slow, plates covered in four to six weeks (Figs. 33–35). Advancing zone even, hyaline and appressed in zone up to 1.0 cm. wide. Mat white or with tinges of pale chalcedony yellow (Ridgway), appressed, downy to woolly-felty, after three to four weeks producing raised balls of mycelium with cottony or velvety surfaces, along radii or scattered. Reverse unchanged. Odour of apples. On gallic acid agar no diffusion zone, on tannic acid agar no diffusion or very weak reaction, diameter 2.0–3.0 cm. on gallic acid agar, no growth on tannic acid agar (Figs. 36, 37) (Group 3).

Advancing zone: hyphae hyaline, nodose-septate, 1.5–3.0(–6.0) μ diameter (Fig. 70). *Aerial mycelium:* (*a*) hyphae as in advancing zone; (*b*) conspicuous much-branched hyphae, the branches usually attached at right angles and frequently rebranched, the walls slightly thickened and rigid, lumina fairly broad and apparently empty, aseptate, 3.0–6.0 μ diameter (Fig. 71); (*c*) conidia numerous, borne singly at the tips of branches that are usually narrower than the main hyphae, about 1.5 μ diameter, thin-walled, broadly ovoid, slightly truncate at distal end, pointed at attached end, 7.0–9.0 $\times$ 4.5–7.0 μ (Fig. 72); (*d*) chlamydospores numerous, intercalary or terminal, walls slightly thickened, broadly ovoid, 9.0–16.5 $\times$ 7.5–12 .0 μ (Fig. 73). The thicker walls and larger size would seem to distinguish chlamydospores from conidia. *Submerged mycelium:* (*a*) hyphae as in advancing zone; (*b*) chlamydospores as in aerial mycelium; (*c*) crystals numerous, octahedral.

Cultures of this species resemble those of *Fomes officinalis* in the abundance of secondary spores, but are readily separated from this and other species by reason of the rigid, thick-walled, much-branched hyphae, which are conspicuous in all preparations for microscopic examination.

No interfertility studies in *Poria carbonica* have been undertaken, partly because the fungus does not fruit in culture and monosporous mycelia must, therefore, be obtained from fruit bodies produced in nature, but mainly because the cultures are so readily recognizable that no additional evidence is necessary to support identification by cultural methods.

History and Nomenclature

During the summer of 1938 a number of collections of this fungus were made in British Columbia by Dr. Irene Mounce. Some of these were submitted to Dr. L. O. Overholts for identification. He was already familiar with the species, and has kindly consented to describe it here.

Poria carbonica Overholts, n. sp. (Figs. 44, 45, 74–76.)

Fructificatio annua, effusa, 3–12 mm. crassa, margine attenuato vel interdum tumido, in substrato verticali plus minus nodulosa, caseoso-lenta in humido, dura in sicco, albida vel isabellina in vetustate, tubulis 2–10 mm. longis, rectis vel obliquis, 3–4 pro mm., dissepimentibus crassis integrisque, dein interdum tenuibus; subiculo distincto, albo, firmo, nec fibroso, 0.5–3 mm. crasso, sporis anguste-ellipsoideis vel fere breve-cylindricis, levibus, hyalinis, 3.5–6.0 × 2.0–3.0 μ; basidiis clavatis 10–12 × 4.5–6 μ; hyphis subiculi 3–4 μ diam., ramosis, nodoso-septatis, muris gelatinosis, hyphis tramae 2–2.5 μ diam., clarioris.

Hab.: in ligno emortuo et saepe carbonario Coniferarum.

"Effused for several centimetres, annual or if reviving for more than one season, at least the tubes not in layers, 3–12 mm. thick, the margin thinning out or at times a bit tumid; on vertical substrata the surface more or less nodulose with the tubes vertical, cheesy-tough when fresh, drying quite hard, white or in age isabelline; tubes 2–10 mm. long, the latter lengths where oblique, the mouths 3–4 per mm., the walls rather thick and entire, at times becoming thin and gaping; subiculum well developed, white, firm, not at all fibrous, 0.5–3 mm. thick; spores narrow-ellipsoid or almost short-cylindric, smooth, hyaline, 3.5–6.0 × 2.0–3.0 μ; basidia clavate, 10–12 × 4.5–6.0 μ; subiculum hyphae with walls considerably gelatinized (or at least unstaining) and often only the lumen visible, 3–4 μ diameter, somewhat branched, with some cross walls and clamps, those of trama 2.0–2.5 μ diameter and more distinct.

"On dead and often charred wood of coniferous trees. Type collected on charred log of *Pinus ponderosa* at Missoula, Mont., September, 1916, J. R. Weir (No. 8198) (Overholts Herb. No. 4169). The following additional collections are in Overholts Herb.: on end of burned log of *Pseudotsuga taxifolia*, Cook Creek, Vancouver Island, B.C., I. Mounce, August 18, 1938 (Dept. of Agr., Ottawa, Mycol. Herb. No. 8444); on *Picea Engelmanni*, Upper Priest River, Idaho, August 1, 1924, C. R. Stillinger (1838); on *Pseudotsuga taxifolia*, Carson, Wash., Oct. 29, 1935, G. H. Englerth (58033); on *Pseudotsuga taxifolia*, Sinslow N.F., Oregon, September 23, 1938, Englerth and Childs (94012); under side of drift log, Kaloma, Wash., October 12, 1909, C. J. Humphrey (5905); on *Tsuga heterophylla*, Revelstoke, B.C., Aug. 26, 1930,

J. R. Hansbrough (40652); on *Pseudotsuga taxifolia* boat timber, Seattle, Wash., May 15, 1942, G. H. Englerth (94160), comm. R. W. Davidson."

Of the 19 collections of *Poria carbonica* in the Dept. of Agr., Ottawa, Mycological Herb., 15 were on rotted and frequently charred logs of *Pseudotsuga taxifolia*, three were on unidentified coniferous logs, and one was on a Douglas fir railway tie. These were all collected in British Columbia, except for one specimen collected near Seattle, Wash., and sent by Dr. J. W. Hotson. In addition, Mr. Ross W. Davidson, of the Division of Forest Pathology, United States Department of Agriculture, has reported (Letter, September 8, 1942) that he has frequently isolated this species from ship timbers.

Acknowledgments

This investigation was possible only because of the generous contribution of specimens and cultures by Sir E. J. Butler, Dr. J. E. Bier, Dr. Lee Bonar, Mr. K. St. G. Cartwright, Mr. R. W. Davidson, Dr. W. P. K. Findlay, Dr. C. W. Fritz, Dr. I. Mounce, Dr. L. O. Overholts, Dr. C. A. Richards, Mr. C. G. Riley, Dr. H. Robak, and Dr. W. H. Snell, to each of whom the author expresses gratitude. She wishes also to thank Dr. L. O. Overholts for describing the new species, Dr. J. W. Groves for preparing the Latin diagnoses, and Dr. H. T. Güssow and Dr. Irene Mounce for their sustained interest in the problem.

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MAIN ARTERIES IN THE NECK AND THORAX OF THE RHEA EMBRYO[1]

BY FRED H. GLENNY[2]

Abstract

A single specimen of a late rhea embryo was dissected and a diagram of the arteries in the neck and thorax prepared. Certain similarities and dissimilarities between the arterial patterns of the rhea, kiwi, and cassowary were noted. The left radix aortae was found to anastomose with the left pulmonary arch, with the subsequent degeneration of the left ductus botalli. The rhea is laevocarotidinae. The right internal carotid becomes functionally modified as do also the ducti carotici.

In April 1941, the writer had the opportunity of studying the arterial arrangement in the neck and thorax of a rhea embryo, *Rhea americana intermedia* (Linné), in the late embryonic stage. Although no definite incubation period could be established, the specimen obviously was very close to the hatching stage—most of the yolk mass being retained (*in corpore*). The embryo was one of the United States National Museum alcoholic specimens.

Routine dissection was carried out, and a diagram of the arrangement-pattern prepared. The arterial arrangement is set forth in the following observations and in Fig. 1.

In the late embryonic stage of the rhea, the left radix aortae (*5*) is found to maintain a proximal connection with the pulmonary (sixth aortic) arch (*6*). From studies on other birds, it is known that this connection results from anastomosis of the anterior portion of the radix (descending aortae) and the proximal portion of the sixth aortic arch (8, 9, 10). This is most readily observed in late embryonic stages of the Canada goose, black-billed cuckoo, Piciformes, and some of the Coraciiformes. After this anastomosis, the distal portion of the sixth aortic arch (ductus arteriosus/ductus botalli) atrophies and either disappears completely or fuses in part with the left radix aortae. Thus the left radix thereafter takes on the function of the ductus botalli (left side) until just prior to or for a very short period after hatching. (The fetal circulation is "short-circuited" by the ductus botalli which permits the flow of blood from the pulmonary arch to the radices aortae. At the same time, there is also pressure—from the blood in the radices aortae—exerted on the blood in the ductus botalli and this in turn exerts a pressure on

[1] *Manuscript received February 26, 1943.*
Contribution from the Department of Zoology, University of Toronto, Toronto, Ont.

[2] *Formerly Assistant, Department of Zoology, University of Toronto; now on active service with the Royal Canadian Army Medical Corps.*

the blood in the pulmonary artery. After obliteration of the left systemic arch, the posterior ramus of the radix aortae anastomoses with the left pulmonary arch, thereby maintaining in part a primitive type of vertebrate circulation. Subsequently the larger vessel (left radix aortae) takes over the

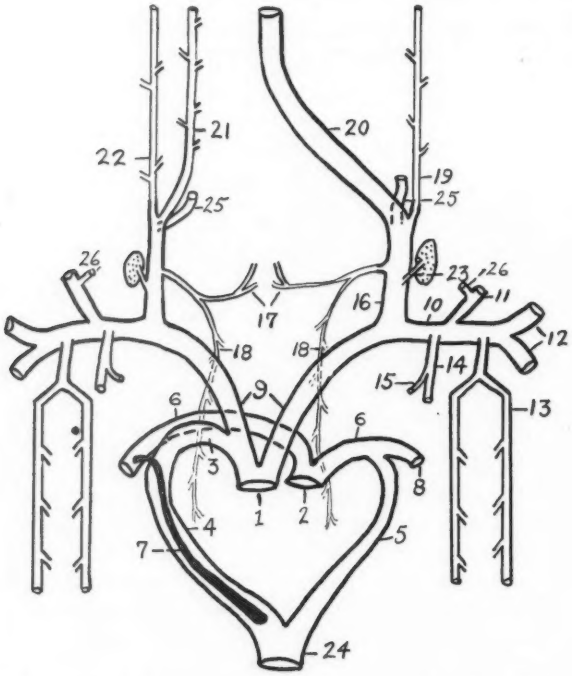

FIG. 1. *Diagrammatic representation of the arteries in the neck and thorax of Rhea americana intermedia, late embryo. Ventral view.*

KEY TO ABBREVIATIONS

(*1*) Aortic root; (*2*) pulmonary root; (*3*) systemic arch; (*4*) right radix aortae; (*5*) left radix aortae; (*6*) pulmonary arch (proximal portion); (*7*) ligamentum botalli (distal portion of sixth arch); (*8*) pulmonary artery; (*9*) innominate arteries; (*10*) subclavian artery; (*11*) axillary artery; (*12*) pectoral arteries; (*13*) intercostal artery; (*14*) coracoid major artery; (*15*) sternotracheal artery; (*16*) common carotid artery; (*17*) syringotracheal arteries; (*18*) ductus shawi; (*19*) left lateral superficial cervical artery; (*20*) left internal carotid (trunk) artery; (*21*) ascending-oesophageal artery; (*22*) right lateral superficial cervical artery; (*23*) thyroid artery; (*24*) dorsal aorta; (*25*) vertebral arteries; (*26*) coracoid minor artery.

normal function of the ductus botalli which, with reduced pressures from both proximal and distal ends, undergoes rapid degeneration. From observations on late embryonic and newly hatched stages of several widely separated species of birds, it appears that the rate and degree of atrophy of the left ductus botalli varies somewhat for the different families and orders of birds.

The factors involved in this are not clearly understood, but are probably based on phyletic relationships and possibly on certain unknown physiological factors of growth and development.)

Shortly after the left ductus botalli atrophies, the right ductus botalli begins to undergo a similar change, but, unlike its homologous structure, does not completely disappear. Instead, it remains as the ligamentum botalli (*7*) and in the adult is probably smaller than the ligamentum aortae.

The right systemic arch (*3*) alone remains as the functional arch of the adult and carries the blood by way of the right radix aortae (*4*) to the dorsal aorta (*24*).

The ductus caroticus becomes functionally modified as in other species of birds after losing its proximal attachment to the radices aortae[1] (Fig. 2). It remains as the ductus shawi (*18*) (*3*) and receives several branches from the trachea and syrinx (*17*), oesophagus, and other tissues dorsal to the heart[2]. Evidences of this have been found in many species of birds, but are most easily demonstrated in members of the Columbidae and in the hairy woodpecker, *Dryobates villosus villosus* L.

The left internal carotid artery (*20*) alone enters the hypapophysial canal and in this respect is not unlike many other species of birds such as the cassowary, kiwi, Piciformes, Passeriformes, and certain families of Coraciiformes (1, 2, 4–6, 8–11). The posterior or proximal length of the right internal carotid (*21*) remains as a superficial artery and appears to be functionally modified to serve as the ascending-oesophageal artery. This also appears to be the case in the kiwi (2, 4), Passeriformes (2, 5), Australian cassowary (6), the Piciformes, and certain families of the Coraciiformes (8, 9).

The innominate arteries (*9*) divide to form the subclavian (*10*) and the common carotid (*16*) arteries. The subclavians give off the coracoid major (*14*), axillary (*11*), intercostal (*13*), and two pectoral (*12*) arteries in order. The common carotids give off the ductus shawi (*18*) and the thyroid (*23*) arteries before dividing to form the internal carotid (trunk) (*20*), lateral superficial cervical (*19* and *22*), and vertebral (*25*) arteries.

The coracoid major sends off a smaller sternotracheal artery (*15*) to supply the sternotracheal muscle, while the axillary artery gives rise to the small coracoid minor artery (*26*). The intercostal artery bifurcates to form a ventral and a lateral branch.

By comparison, the general basic arrangement-pattern in the rhea is not entirely dissimilar to that of the kiwi and the Australian cassowary, but in some respects each is to be regarded as singular.

[1] "*. . . At the same time, the left ductus caroticus (dorsal portion of the radix aortae between the 3rd and 4th aortic arches) loses its proximal connection in the posterior ramus of the radix (due to the obliteration of the left 4th aortic arch) and becomes functionally modified thru subsequent anastomosis with several small arteries which form in situ and subsequently come to supply the oesophagus, trachea, syrinx, and other tissues of the thoracic cavity, dorsal to the heart.*" (*9*).

[2] "*. . . anastomoses of the ductus caroticus with syrinx, tracheal, and oesophageal arteries which form in situ to give rise to the ductus shawi (Glenny, 1940b, 1942a, 1942c) are quite common and are expected in birds.*" (*8*).

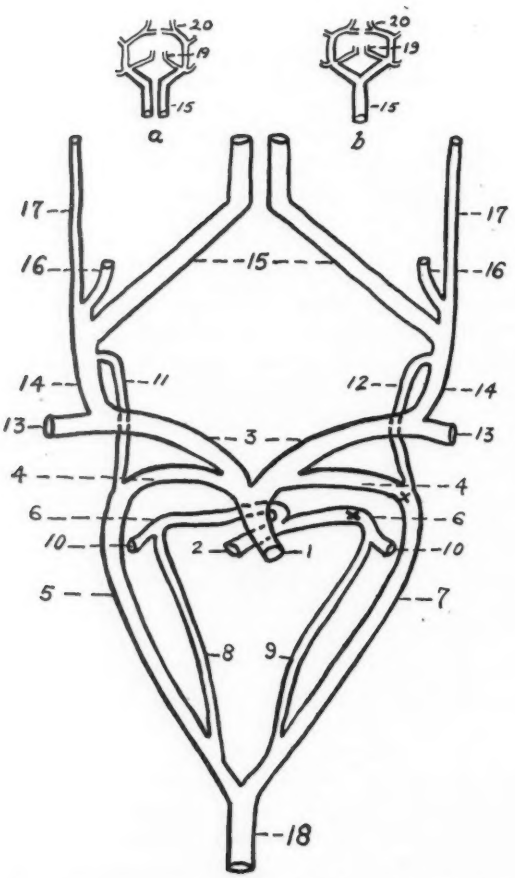

FIG. 2. *Diagrammatic representation of the aortic arches and associated main arteries in the neck and thorax of birds (to illustrate some of the changes that take place during middle and late embryogeny). Ventral view.*

Two small diagrams at top of page—cephalic branching of internal and external carotid arteries: (a) bicarotidinae normales; (b) laevocarotidinae.

KEY TO ABBREVIATIONS

(*1*) Aortic root; (*2*) pulmonary root; (*3*) innominate artery; (*4*) systemic (fourth aortic) arch; (*5*) right radix aortae (aorta descendens); (*6*) pulmonary (sixth aortic) arch; (*7*) left radix aortae; (*8*) right ductus botalli; (*9*) left ductus botalli; (*10*) pulmonary arteries; (*11*) right ductus caroticus; (*12*) left ductus caroticus; (*13*) arteria subclavia secunda; (*14*) ventral aorta; (*15*) internal carotid (trunk) artery (adult position); (*16*) vertebral arteries; (*17*) definitive or adult superficial cervical arteries; (*18*) dorsal aorta; (*19*) external carotid (maxillary) artery; (*20*) internal carotid artery; (*x*) marks the approximate position of the anastomosis between the left radix aortae and the left pulmonary arch (proximal portion).

Points of Similarity:

1. Left internal carotid (trunk) artery alone enters the hypapophysial canal—to carry the cephalic blood supply.

2. Right internal carotid (trunk) artery becomes functionally modified as the primary ascending-oesophageal artery.

3. Left radix aortae remains as a prominent ligamentous vestige of the embryonic vessel—ligamentum aortae.

4. Ductus caroticus is functionally modified to form the ductus shawi—several smaller vessels which form *in situ* anastomose with the ductus caroticus prior to or just after it loses its proximal connection with the systemic arch.

Points of Dissimilarity:

1. Major branches of the subclavian arteries vary in relative position and in supply (in the kiwi and cassowary) from the general avian pattern.

2. Secondary vessels differ in points of origin, number, and supply.

3. Relative position (origin) of the internal carotid (trunk), vertebral, and superficial cervical arteries is different in each of the three species.

4. In *Casuarius australis* the right cervico-intercostal artery sends off a dorsal intercostal artery and several pairs of short segmentally arranged dorsal intercostal and cervical twigs before becoming the right vertebral artery.

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DRIED WHOLE EGG POWDER

II. EFFECT OF HEAT TREATMENT ON QUALITY[1]

By W. Harold White[2] and M. W. Thistle[2]

Abstract

The effect on quality of heating dried egg powders from two Canadian plants at temperatures ranging from 26.7° to 60.0° C. for periods of from three hours to seven days was investigated. Quality of the treated powders was assessed by determination of the fluorescence, potassium chloride, and water values and the pH and refractive indices of the potassium chloride and aqueous extracts.

The rate of deterioration of quality was on the average usually greatest on heating for one day at temperatures of 43.3° C. and higher. However, some change was observed even at 26.7° C. after one day. The powders from the two plants behaved similarly. Interpretation of the results in terms of the rate at which egg powder should be cooled after drying indicated that a temperature of 26.7° C. or less should be attained within three hours if deterioration in quality is to be prevented.

Introduction

Until recently dried whole egg powder was usually considered to be a relatively stable food product. Accordingly little attention has been given to the temperature conditions to which dried egg is exposed after its manufacture. However, experience with other foodstuffs has shown that temperature is usually one of the prime factors determining the rate of deterioration. Examination indicated that deterioration of dried egg might result either from the relatively high temperatures of the powder when removed from the drier and the extended period required for it to cool in commercial packages or from subjection to unfavourable temperature conditions during storage and transportation. The present paper deals with the effect on quality of temperatures comparable with those to which dried whole egg powder may be subjected on removal from commercial driers. Studies of the effect of storage temperature and other factors, such as the moisture content and gas packing, will be described in subsequent papers.

Material and Procedure

Egg powder was obtained from two plants, selected to represent the two main types of drying practice used in Canada. In Plant I, Grades B and C storage eggs were dried in a box type drier at an inlet temperature of 149° C. (300° F.) and an outlet temperature of about 63° C. (145° F.). In Plant II, Grades B and C storage eggs were dried in a cone type drier at approximately the same temperatures as noted above.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Material selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as paper No. 90 of the Canadian Committee on Food Preservation and as N.R.C. No. 1125.

[2] *Biochemist, Food Investigations.*

Immediately upon removal from the drier the powder was placed in rubber latex bags, and cooled to a temperature of 0° C. The powder was shipped from the plants in special containers at a temperature of 0° C. Upon arrival at the laboratory, 100-gm. samples were sealed in tin cans in a room at 4° C. (40° F.). It was considered that these precautions were sufficient to prevent any change in the quality from the time of collection of the powder to its treatment in the laboratory.

The specially collected powders from each of the two plants were heated rapidly to, and maintained at, temperatures of 26.7°, 35.0°, 43.3°, 51.7°, and 60.0° C. (80°, 95°, 110°, 125°, and 140° F.). Samples were removed for analysis after heating for 3, 6, and 12 hours and one, two, three, four, five, six, and seven days, and immediately cooled to 0° C. The selection of temperatures was based on the following considerations: 35.0°, 43.3°, and 51.7° C. represented approximately the minimum, average, and maximum temperatures of the powders on leaving the driers, as observed in Canadian plants; 26.7° C. was considered to be a temperature to which powder could be cooled without excessive practical difficulty; and 60.0° C. was selected to determine the effect of an abnormally high temperature on the quality of dried egg.

The quality of the powder after various heat treatments was assessed by determination in duplicate of the fluorescence, water, and potassium chloride values. The methods employed have been described previously (2, 3). The fluorescence value gives a measure of the overall quality of dried egg and is related to flavour quality (2). The potassium chloride and, to a lesser extent, the water values are related to overall quality, and also indicate the solubility of egg powder (3).

In addition, the pH and refractive indices of the potassium chloride and aqueous extracts were determined, since it was considered that they might bear some relation to quality. Measurements of the pH were made at 25° C. on residual solutions from the potassium chloride and water value determinations with a Beckman pH meter, using a glass electrode and a saturated calomel half-cell. Refractive indices of the same solutions were measured in an Abbé refractometer at 25° C.

Results

Analyses of Variance

The relative importance of temperature, time of heating, and source of the egg powder in affecting the quality was determined by means of analyses of variance. The results (Table I) show that temperature had the greatest effect on quality, as assessed by all the measurements. However, differences that were attributable to variations in the time of heating and the plant from which the egg powder was obtained usually attained statistical significance.

Mean values for each variable, as calculated over all others for the whole experiment, are given for each measurement in Table II. It is to be noted that the data obtained for the refractive indices of the potassium chloride

TABLE I

ANALYSES OF VARIANCE FOR THE EFFECT OF HEAT TREATMENT ON THE QUALITY OF DRIED EGG POWDERS, AS ASSESSED BY VARIOUS PHYSICAL AND CHEMICAL MEASUREMENTS

Source of variance	D.f.	Mean square						
		Fluorescence value	KCl value	Water value	pH of KCl extract	pH of aqueous extract	Refractometric value of KCl extract	Refractometric value of aqueous extract
Temperature	4	65,907**	16,388**	12,741**	8.6530**	5.1132**	2284.0**	1544.4**
Time	10	17,800**	3974.4**	1834.6**	2.6008**	0.9703**	489.23**	337.02**
Plants	1	5650.4**	7401.9**	1196.2**	1.3904**	0.3496**	868.04**	29.823
Temperature × time	40	3390.1**	706.13**	657.82**	0.3113**	0.2929**	113.93**	83.763**
Plants × temperature	4	459.21**	61.777	300.02*	0.0342	0.0296	5.3818	53.061*
Plants × time	10	195.53**	22.955	220.78*	0.0176	0.0608	9.7209	41.473**
Residual	40	60.811	34.042	87.718	0.0133	0.0152	9.6118	14.324
Duplicate error	110	1.5961	0.8703	1.4787	0.0021	0.0024	1.9227	3.2318

* *Exceeds 5% level of statistical significance.*
** *Exceeds 1% level of statistical significance.*

TABLE II

MEAN VALUES FOR THE EFFECT OF HEAT TREATMENT ON THE QUALITY OF DRIED EGG POWDERS, AS ASSESSED BY VARIOUS PHYSICAL AND CHEMICAL MEASUREMENTS

Factor	Fluorescence value[1]	KCl value[1]	Water value[1]	pH of KCl extract[1]	pH of aqueous extract[1]	Refractometric value of KCl extract[1]	Refractometric value of aqueous extract[1]
Temperature, ° C.							
26.7	16.5	78.5	74.8	8.39	8.44	31.0	28.5
35.0	22.3	76.6	77.5	8.28	8.39	29.5	29.7
43.3	36.8	60.3	70.2	8.01	8.23	24.0	27.3
51.7	82.2	42.9	48.7	7.61	7.90	17.6	20.2
60.0	104.0	35.7	39.4	7.34	7.64	14.5	16.0
Necessary difference[2]	3.4	2.5	4.0	0.05	0.05	1.3	1.6
Time, days							
0	13.8	76.4	69.9	8.45	8.40	29.2	30.8
0.13	14.3	80.4	72.1	8.38	8.36	30.0	28.0
0.25	21.5	76.6	75.7	8.36	8.37	30.4	28.1
0.50	32.3	68.1	75.5	8.19	8.30	25.0	28.4
1	42.1	59.0	61.8	8.00	8.25	25.5	25.2
2	49.0	53.8	59.1	7.83	8.07	21.9	23.6
3	62.8	49.2	58.6	7.67	8.04	20.0	23.6
4	76.3	48.9	54.6	7.64	7.95	20.6	21.4
5	86.6	45.7	51.9	7.57	7.85	18.0	20.4
6	88.6	44.8	53.8	7.56	7.90	18.0	20.4
7	88.8	43.8	50.3	7.57	7.83	17.9	17.8
Necessary difference[2]	5.0	3.7	6.0	0.07	0.08	2.0	2.4
Plant							
I	47.3	64.6	64.5	8.01	8.16	25.3	24.7
II	57.4	53.0	59.8	7.85	8.08	21.3	24.0

[1] *Mean values for all other conditions over the whole experiment.*
[2] *Necessary difference required to exceed 5% level of statistical significance.*

and aqueous extracts have been reduced for the sake of convenience to the form of "refractometric values", i.e. (refractive index of extract − refractive index of solvent) × 10^4. In general, differences between temperatures were usually least between 26.7° and 35.0° C. and greatest between 43.3° and 51.7° C. The greatest change with time occurred usually during the first day. The powder from Plant I deteriorated at a slower rate than that from Plant II. This difference is considered to be due primarily to the fact that the powder from Plant I contained 3.5% moisture while that of Plant II contained 5.6%. It has been found that the stability of egg powder decreases with increase in the moisture content. Details of this investigation are to be published in the near future.

Details of the changes in the fluorescence and the potassium chloride values, and the pH and refractometric values of the potassium chloride extracts are graphically illustrated in Figs. 1 to 4, respectively. Curves for the corresponding measurements on aqueous extracts were in general similar to those given.

Fluorescence Value

An increase in either the temperature or time of heating caused an increase in the fluorescence values (Fig. 1). The rate of formation of fluorescence materials was relatively great at all temperatures above 26.7° C. However, even at this temperature, deterioration in quality occurred if the powder was heated for more than three hours.

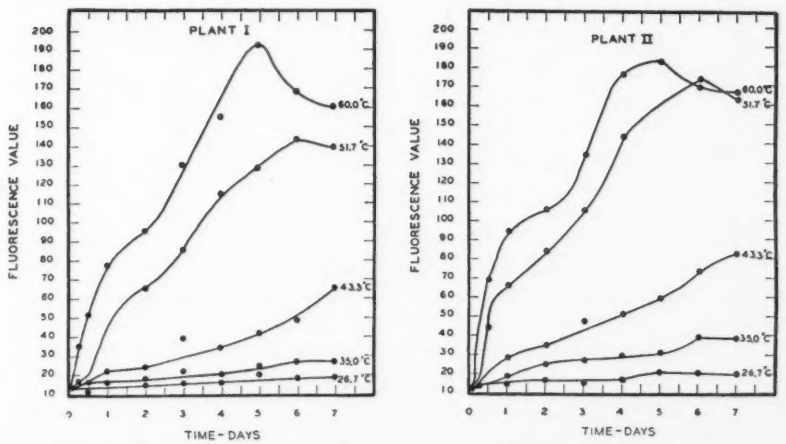

FIG. 1. *Effect of heat treatment on the fluorescence values of dried egg powders.*

The results of a recent investigation indicate that the fluorescent materials are formed by hydrolysis of the protein fraction of egg powder (1). It is of some interest to examine the present data with respect to the nature of the reactions producing the fluorescent materials. The general conformity of the curves (Fig. 1) for the two plants suggests that similar types of reactions

were occurring in both powders regardless of their method of preparation. At 51.7° and 60.0° C. there is some evidence that two or more reactions yielded fluorescent end products. Since there was some indication that the fluorescence values decreased after reaching a maximum, it would seem that at these two temperatures the reactions had reached completion, and that fluorescent materials were being thermally decomposed.

Potassium Chloride and Water Values

The potassium chloride (Fig. 2) and water values of the powders decreased on heating, indicating thermal decomposition of the fat–protein complex and denaturation of the egg protein. The most marked decreases were observed at 51.7° and 60.0° C. on heating for one day. Subsequent changes

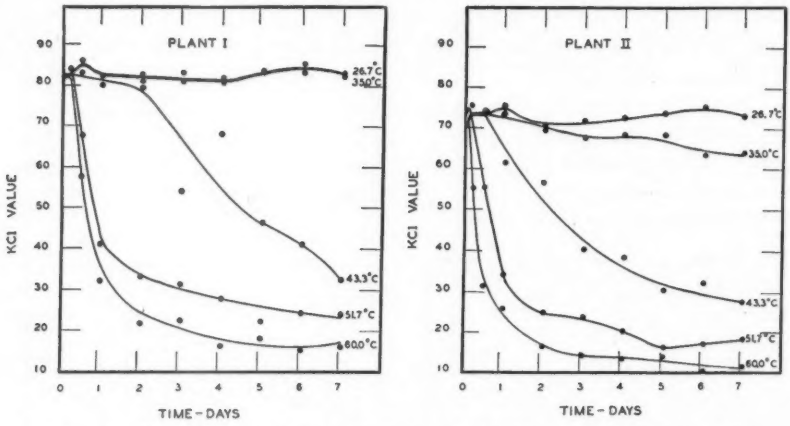

FIG. 2. *Effect of heat treatment on the potassium chloride values of dried egg powders.*

at these temperatures were relatively slow, indicating that the denaturation reactions were approaching completion. The decreases observed at 26.7° and 35.0° C. were relatively small and approximately of the same order of magnitude. The reason for the slight increase in potassium chloride and water values for the shorter heating periods is unknown. However, it may be that the nature of the fat–protein complex is altered, thereby permitting more fat to pass through the filter paper.

In actual magnitude the water values were lower initially than the potassium chloride values, and changed less during the experiment. This behaviour suggests that not only could more of the egg powder be dissolved or dispersed in 10% potassium chloride solution than in water, but that these materials were more unstable thermally. Moreover, the water values were rather variable and decreased appreciably with increase in the time of heating only at temperatures of 43.3° C. and higher. Thus, the potassium chloride value is a more sensitive and precise measurement of quality than the water value.

In general the potassium chloride and water values were less sensitive to the effects of heat treatment than the fluorescence value.

pH of Potassium Chloride and Aqueous Extracts

The pH of the potassium chloride (Fig. 3) and aqueous extracts decreased as the temperature and time of heating were increased, indicating that the thermal decomposition products were acidic in character. The greatest changes usually occurred during the first day and at temperatures of 51.7° and 60.0° C. The data for the pH of aqueous extracts were more variable than those for the potassium chloride extracts and the magnitude of the changes was smaller. This is in agreement with previous observations on potassium chloride and water values.

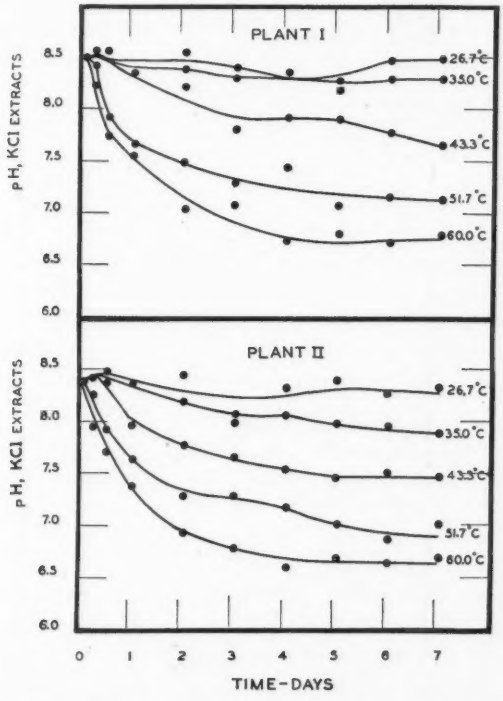

FIG. 3. *Effect of heat treatment on the pH of potassium chloride extracts of dried egg powders.*

Refractometric Values of Potassium Chloride and Aqueous Extracts

The refractometric values of the potassium chloride and aqueous extracts were somewhat small because of the relatively dilute extract of egg powder used, and are consequently subject to a relatively large experimental error in

measurement with the Abbé refractometer. In spite of this the determination was sufficiently sensitive to detect changes similar to those observed for the other measurements on these extracts (cf. Figs. 2, 3, and 4). An increase in either the temperature or time of heating caused a decrease in the refractometric values for both plants. The values for Plant I were usually higher at all temperatures and times of heating.

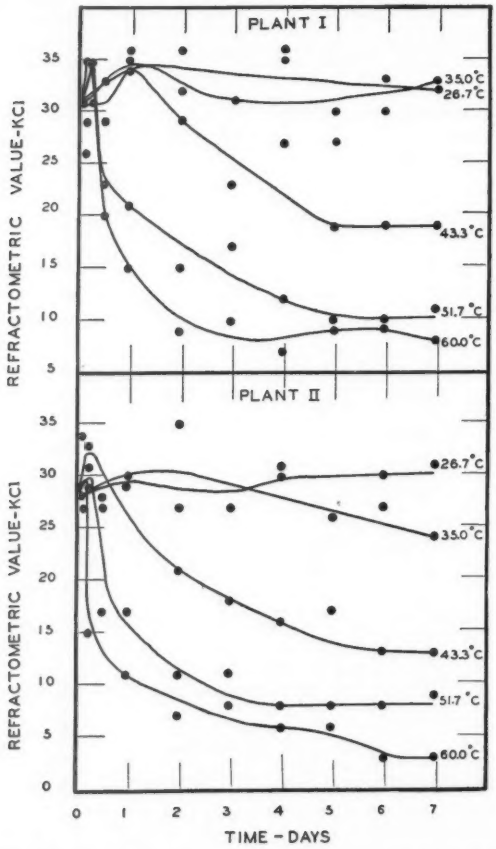

FIG. 4. *Effect of heat treatment on the refractometric values of potassium chloride extracts of dried egg powders.*

Interrelation of Methods for Assessing Quality

It is apparent from the preceding discussion that the methods employed for assessing quality were more or less related. The degree of interrelation was determined by the computation of simple coefficients of correlation. Data were used for only those samples that corresponded to egg powder of

suitable quality for export by present Canadian standards, i.e. a fluorescence value of 52 or lower and a potassium chloride value of 40 or higher. Even within these limits the coefficients are possibly unsuitable for prediction purposes because of a higher proportion of low grade material than would be obtained in Canadian egg powders prepared for export. The fluorescence and potassium chloride values, and the pH and refractive index of potassium chloride extracts would appear to be the most suitable of the methods studied for estimating deterioration in the quality of egg powder as a result of adverse heat treatment (Table III).

TABLE III

COEFFICIENTS OF CORRELATION BETWEEN VARIOUS MEASUREMENTS OF QUALITY OF DRIED WHOLE EGG POWDER

Methods	Methods					
	pH of water extract	pH of KCl extract	Refractometric value of aqueous extract	Refractometric value of KCl extract	Water value	KCl value
Fluorescence	−0.62**	−0.86**	−0.24*	−0.78**	−0.14	−0.84**
KCl value	0.66**	0.88**	0.26*	0.84**	0.22	—
Water value	0.33**	0.16	0.64**	0.11	—	—
Refractometric value of KCl extract	0.69**	0.81**	0.17	—	—	—
Refractometric value of aqueous extract	0.45**	0.22	—	—	—	—
pH of KCl extract	0.62**	—	—	—	—	—

** Exceeds 5% level of statistical significance for the 68 degrees of freedom available.*
*** Exceeds 1% level of statistical significance for the 68 degrees of freedom available.*

Discussion

While the investigation was concerned with the effect of heat treatment on the quality of egg powder, the results can be interpreted in terms of the rate and temperature to which dried egg should be cooled as it comes from the drier. Of the conditions studied, deterioration in quality occurred if powder was heated at temperatures of 35.0° C. and higher for three hours, whereas there was little change at 26.7° C. in this period. Hence, it may reasonably be concluded that dried egg should be cooled to 26.7° C. or lower within three hours after removal from the drier.

This conclusion has been confirmed by practical tests in Canadian egg drying plants. In these the quality of promptly cooled powder was compared with that of the same powder treated by the normal practices of the particular plant. Results obtained for two plants are given in Table IV. The effectiveness of rapid cooling in maintaining quality is obvious.

The marked thermal instability of dried whole egg powder, as demonstrated in the experimental investigation and confirmed in commercial practice, has

TABLE IV

COMMERCIAL TESTS ON THE EFFECT OF RAPID COOLING ON THE QUALITY OF CANADIAN DRIED EGG POWDER

Plant	Normal cooling		Rapid cooling	
	Fluorescence value	Predicted flavour score[1]	Fluorescence value	Predicted flavour score[1]
A	29.2	7.1	14.8	9.6
B	25.2	7.7	13.3	9.9

[1] *Computed from the equation given by Pearce and Thistle* (2).

led to the adoption of a regulation requiring that all Canadian egg powders prepared for export to England be cooled to a temperature of 26.7° C. or lower within one hour from the time of their formation. It is considered that rigid adherence to this regulation will materially raise the general level of quality of Canadian dried egg powder.

Acknowledgments

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DRIED WHOLE EGG POWDER

III. A REFRACTOMETRIC METHOD FOR THE DETERMINATION OF SOLUBILITY[1]

By W. Harold White[2] and G. A. Grant[3]

Abstract

The refractometric determination of the solubility of dried, whole egg powder was found to be affected by the method of defatting, the nature of the fat and protein solvents, the ratio of protein solvent to powder, the method of equilibration, and time of extraction. These factors were standardized by defatting egg powder with petroleum ether and extracting for a period of two hours with a 5% solution of sodium chloride. The refractometric value of the extract was determined at 25° C. using an Abbé refractometer.

The refractometric value was linearly related to the content of water soluble nitrogen of whole powder and to the potassium chloride value of defatted powder. A curvilinear relation was obtained with the content of crude albumin nitrogen and with the potassium chloride value of whole egg powder.

Introduction

Egg powders of edible quality may be divided into two groups, namely, those fit for consumption as egg dishes, and those satisfactory only for use by bakers or other food manufacturers requiring a source of edible, soluble protein for their products. Hence, solubility is a useful criterion for grading egg powders. The present paper describes a rapid and convenient refractometric method for determining the solubility of egg powder.

None of the known methods for the determination of the solubility of egg powder are entirely satisfactory. Methods involving the Kjeldahl determination of nitrogen (2, p. 309, 4) are somewhat time consuming, and depend on obtaining a clear extract from the egg powder. For the most part, the potassium chloride value (6) gives a measure of overall quality rather than of solubility alone. A method based on the determination of the volume of the water soluble, heat-coagulable protein (5) was considered to be somewhat insensitive. In these circumstances attention was given to other possible procedures for determining solubility. Of these, refractometric methods have been successfully used for the determination of the solids content of shell eggs (1, 3, 7). The results of a preliminary study indicated that they offered possibilities of suitable adaptation to dried egg (8).

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Material selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, Montreal, Que., in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as Paper No. 91 of the Canadian Committee on Food Preservation and as N.R.C. No. 1126.

[2] *Biochemist, Food Investigations.*

[3] *Laboratory Assistant, Food Investigations.*

Procedure and Results

Fat Extraction

A preliminary study was made of the relative suitability of using whole or defatted egg powder for the determination. For this purpose 1 gm. samples of whole egg powder were extracted with 5 ml. of a 5% solution of sodium chloride for 30 min. No marked separation of the liquid and solid phases occurred except in powders of poor quality. This made accurate determination of the refractive index of the extract in the Abbé refractometer difficult. Removal of the fat prior to extraction gave a much clearer extract, and it appeared that extraction of the protein would be facilitated by the removal of the fat fraction. Attempts to extract the fat and soluble protein with mixed solvents in one step were unsuccessful, indicating that the two treatments should be made separately.

The effect of defatting was investigated on four samples of egg powder known to differ in their contents of soluble protein. Four grams of each powder were defatted by manual shaking at room temperature with four 50 ml. portions of petroleum ether (boiling range 30 to 60° C.), and filtered each time through a No. 1 Whatman filter paper. The defatted powders were allowed to stand about 30 min. at room temperature to permit the evaporation of residual solvent. Both the whole and defatted powders were extracted by shaking 1.00 gm. with 5 ml. of 10% potassium chloride solution at three 10 min. intervals. The refractive indices of a small portion of the extracts were determined at 25° C. in an Abbé refractometer.

The results, given in Table I, are expressed as "refractometric values", that is (refractive index of extract − refractive index of solvent) × 10^4. With the exception of one sample the refractometric values for defatted powder were higher than those for whole powder. This was to be expected because of the greater amount of protein material present in defatted powder. Thus, in addition to giving extracts that can be more easily read in the Abbé refractometer, the use of defatted powder permits a relatively more accurate and precise determination of solubility by virtue of the larger readings obtained.

TABLE I

EFFECT OF DEFATTING AND OF THE SOLVENT USED FOR EXTRACTION OF PROTEIN ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Whole powder		Defatted powder		
1 gm. samples		0.6 gm. samples	1 gm. samples	
5% NaCl	10% KCl	10% KCl	5% NaCl	10% KCl
225	220	150	229	206
192	177	147	217	209
120	112	115	176	170
53	49	49	82	76

* *Expressed as (refractive index of extract − refractive index of protein solvent) × 10^4 at 25° C.*

The difference in refractometric values of 1 gm. of whole and defatted powder was smaller than expected for the two samples of prime quality. This suggested that a portion of the egg powder that is normally capable of being dissolved in 10% potassium chloride solution was removed by defatting. Accordingly refractometric values were determined on 0.6 gm. of defatted powder, an amount approximately equivalent to the non-lipid fraction of 1 gm. of whole powder. The values obtained (Table I) were smaller than those for an equivalent amount of whole powder for the first two samples, and approximately the same for the two heat denatured samples. This suggests the presence in egg powder of a fat–protein complex that can be dispersed or dissolved in 10% potassium chloride solution or petroleum ether, and that is decomposed by heat (6).

As a result of the above observations, the relative suitability of various solvents for defatting egg powder was investigated. For this purpose, four samples of egg powder were defatted by the method described previously with each of the solvents, petroleum ether, acetone, chloroform, and benzene. One gram samples of the defatted powder were shaken with 5 ml. of a 5% solution of sodium chloride for two hours, and the refractive index measured in the Abbé refractometer. A 5% sodium chloride solution was used on the basis of the results of a simultaneous study on protein solvents.

The refractometric values (Table II) for samples defatted with petroleum ether or acetone were approximately of the same magnitude, and lower than those for powders treated with chloroform or benzene. The use of petroleum ether or chloroform gave slightly more precise results. Petroleum ether was selected as the most suitable solvent of those studied on the basis of such considerations as its ease of removal from the defatted powder, and its relatively non-toxic character. Moreover, since the test was to be used for routine quality control, it was desirable to avoid the tedious filtering procedure. The use of petroleum ether rather than chloroform made centrifuging possible.

TABLE II

EFFECT OF SOLVENT USED FOR DEFATTING ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Petroleum ether	Acetone	Chloroform	Benzene
250	251	255	258
229	225	228	239
214	212	222	226
144	143	163	156

* *As defined in Table I.*

The suitability of separating the powder and solvent by centrifuging was investigated. Four-gram portions of each of four different egg powders were weighed into 50 ml. centrifuge tubes, 40 ml. of petroleum ether added, the

mixture stirred, then centrifuged for approximately five minutes, and the liquid decanted. This procedure was repeated three times. The same four samples were defatted by the filtering procedure previously described. Refractometric values were determined, using 5% sodium chloride solution.

Refractometric values obtained for samples defatted by these two procedures are given in Table III. The centrifuging method was selected as being the more suitable since it was more rapid and convenient experimentally, and moreover, gave slightly higher results. The reason for this latter behaviour is not apparent. It was subsequently considered that the number of fat extractions could be reduced to two without materially affecting the results.

TABLE III

EFFECT OF METHOD OF DEFATTING ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Method of defatting		Method of defatting	
Filtering	Centrifuging	Filtering	Centrifuging
257	265	221	229
233	244	143	146

* *As defined in Table I.*

Protein Extraction

The relative suitability of two protein solvents, namely, a 5% solution of sodium chloride and a 10% solution of potassium chloride (4) was studied on four different samples of whole and defatted powder. The results obtained are given in Table I. The refractometric values with the sodium chloride solution were higher than with the potassium chloride solution for both whole and defatted powder. Moreover, the sodium-chloride–soluble components of defatted powder appeared to be somewhat more prone to heat denaturation since the range of values between samples of good and poor quality was greater. A 5% solution of sodium chloride was accordingly selected as being the more suitable solvent. Since the extract obtained with this solvent from defatted powder gave sufficient sensitivity to the determination as a whole, it appeared that further comparison of protein solvents was unnecessary.

The effect of extraction with various amounts of sodium chloride solution on the refractometric value was studied on four samples of egg powder. To 1 gm. of defatted egg powder were added 5, 10, 15, or 25 ml. of 5% sodium chloride solution. The samples were mechanically mixed for two hours and the refractometric values determined at 25° C. in the Abbé refractometer. The results, given in Table IV, show that as the ratio of solvent to powder is increased, the refractometric values decrease. Since the samples treated with 5 ml. of solvent gave the highest values and the greatest difference

TABLE IV

EFFECT OF VOLUME OF SOLVENT USED FOR EXTRACTION OF PROTEIN ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Volume of 5% NaCl solution, ml.			
5	10	15	25
260	130	82	50
236	123	80	47
243	118	79	42
102	40	23	20

* *As defined in Table I.*

between samples of poor and good quality, this volume was selected as being the most suitable of those studied.

One of the most important steps in the determination of solubility of egg powder is the method employed for equilibrating the dried egg with the solvent. The relative effectiveness of a number of possible methods was studied. In the first of these investigations four samples of defatted egg powder, to which 5 ml. of a 5% solution of sodium chloride had been added, were shaken both by hand and in a mechanical shaking machine. When shaken by hand the test-tubes containing the samples were inverted 10 times at four equal intervals during the periods studied.

Refractometric values of the four samples were: 253, 233, 185, and 89 with mechanical shaking, and 235, 229, 179, and 90 with manual shaking. It would appear that mechanical shaking was more effective than the manual method employed. It was found also that solution of the protein was aided by the presence of glass beads in the test-tube during mechanical shaking. However, visual observation indicated that more effective mixing of the solvent and powder would be desirable.

The apparatus shown in Fig. 1 was found to give thorough mixing of the solvent and powder. When the test-tube is shaken at a rate of approximately 300 impulses per minute, the glass rod fastened to the lower end of the flexible rubber tubing moves back and forth rapidly in the tube, thoroughly mixing the contents. It was found that shaking at higher speeds gave an extract that was difficult to read in the Abbé refractometer.

The time required for solution was studied next, using four samples of egg powder. After defatting by the centrifuge method described previously, 1 gm. samples were weighed into test-tubes, 5 ml. of 5% sodium chloride solution added, and the samples mixed by the method described above. Refractive indices of the extracts were determined after shaking for periods of 0.5, 1, 2, and 5 hours.

The results (Table V) show that equilibrium had for the most part been reached after shaking for two hours, although there was some indication that

complete solution of the soluble constituents of high quality powder may require periods of five hours or longer. However, since the differences were small, prolongation of the extraction period beyond two hours usually did not appear to be warranted.

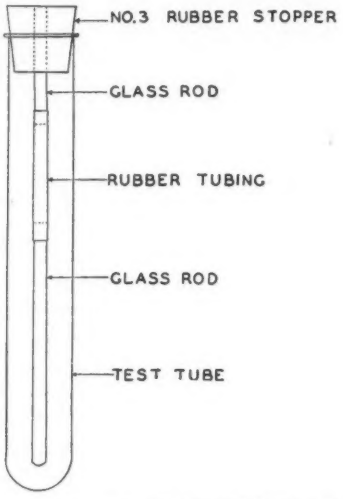

FIG. 1. *Diagram of apparatus employed for equilibrating dried egg powder with the protein solvent.*

TABLE V

EFFECT OF PERIOD EMPLOYED FOR EXTRACTION OF PROTEIN ON THE REFRACTOMETRIC VALUES* OF FOUR EGG POWDERS

Extraction period, hr.			
0.5	1.0	2.0	5.0
215	222	244	253
175	202	208	211
167	176	175	178
78	76	78	84

* *As defined in Table I.*

Suggested Procedure for the Determination of the Refractometric Value

On the basis of the above results, the following procedure was selected as being the most suitable for determining the solubility of egg powder refractometrically. Approximately 4 gm. of egg powder was weighed into a 50 ml. centrifuge tube, mixed thoroughly with 40 ml. of petroleum ether (boiling range 30 to 50° C.), and centrifuged. After decanting the liquid, the procedure was repeated a second time. The powder was spread out on a filter paper to dry at room temperature for about 30 min. One gram of the

defatted powder was accurately weighed into a test-tube (22 × 175 mm.) and 5 ml. of a 5% solution of sodium chloride added. The glass rod of the mixing device was carefully centred vertically in the tube and the tube mechanically shaken at approximately 300 impulses per minute for two hours. A drop of the extract, removed from the test-tube by means of a piece of small diameter, glass tubing, was placed in the Abbé refractometer and the refractive index determined at 25° C.

Interrelation with Other Solubility Tests

It was of interest to compare the refractometric with other known procedures for determining the solubility of egg powders. Determinations of the potassium chloride values, water soluble nitrogen, and crude albumin nitrogen were selected for this purpose. Duplicate determinations were made by each method on 12 samples of egg powder, six of which were products of prime quality, secured from five different Canadian egg driers, while the remaining six were powders secured from two plants and heat-denatured to various extents.

Potassium chloride values were determined on whole powder by a method described previously (6). The part contributed by the fat–protein complex on this determination was also studied by carefully defatting the usual amount of powder employed (2 gm.) prior to extraction with a 10% solution of potassium chloride. The contents of water soluble and crude albumin nitrogen were determined by the tentative standard methods of the A.O.A.C.

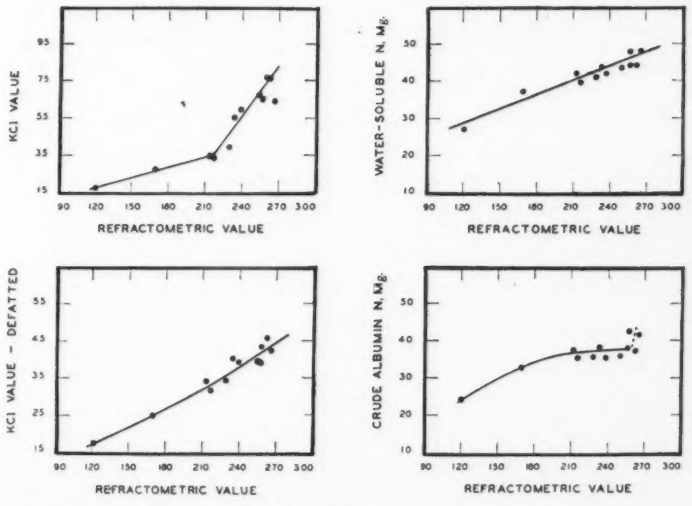

FIG. 2. *The relations between the refractometric value and the potassium chloride values of whole and defatted powder, and the contents of water soluble and crude albumin nitrogen.*

(2). It should be mentioned that some difficulty was encountered in obtaining clear filtrates in the determination of water soluble nitrogen.

The results are shown graphically in Fig. 2. For the rather limited number of samples studied, the refractometric values are for the most part linearly related to the potassium chloride values of defatted powder, and to the content of water soluble nitrogen. A curvilinear relation was obtained for crude albumin nitrogen. The relation between refractometric values and potassium chloride values of whole egg powder is of particular interest. It would appear that potassium chloride values of approximately 35% and higher give a measure of both the amount of soluble protein and of the fat–protein complex present. However, values below about 35% are approximately the same for both whole and defatted powder, indicating that the potassium chloride value in this range is dependent only on the amount of soluble protein present. The fat–protein complex had apparently been thermally decomposed or so altered that the fat could not pass through the filter paper.

Conclusion

The refractometric method described has been successfully used for the determination of the soluble protein content of a large number of samples of egg powders in these laboratories. It was found to be sufficiently precise, rapid, and otherwise suitable for use both in research investigations and routine quality control.

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DRIED WHOLE EGG POWDER

IV. EFFECT OF MOISTURE CONTENT ON KEEPING QUALITY[1]

By W. Harold White[2] and M. W. Thistle[2]

Abstract

Dried whole egg powders, obtained from three different manufacturers, were adjusted to contain from 2 to 8.5% moisture, and held at temperatures ranging from 7.1° to 43.3° C. Quality was assessed by determination of the fluorescence, potassium chloride, and refractometric values.

Temperature was the most important single factor studied in affecting the keeping quality of dried egg. However, at all temperatures the rate of deterioration increased with increase in the moisture content. To maintain quality during storage and transport, dried egg should contain not more than 5% moisture and preferably 2% or less.

Introduction

Moisture content is usually an important factor in the preservation of dried foods. A low moisture content in dried eggs is normally associated with poor initial quality as a result of too severe drying conditions, unless suitable precautions are taken. At moisture levels of about 8% and higher deterioration due to the growth of moulds has been observed (1). Practical experience has indicated that 5% is a suitable maximum content, but there are few scientific data to confirm this opinion. Consequently it was of importance to investigate the effect of moisture content on quality retention in dried whole egg powder held at various temperatures.

In a recent investigation it was found that the solubility of egg powder, stored at 30° C. and at various relative humidities, decreased less at ultimate moisture contents of about 5% and lower than at the higher levels studied (3). However, this type of experiment suffers from the disadvantage that the moisture content of the powder changes during storage, and in consequence, it is difficult to assess the effect on quality of any particular moisture level. Furthermore, the possibility of a differential effect of moisture content with temperature was not studied.

The present investigation was divided into two parts. In an initial experiment the behaviour of egg powder, adjusted to three moisture levels, was studied during storage at temperatures to which egg powder might normally be exposed. In the main experiment a more extensive series of moisture levels was employed to permit definite conclusions to be made concerning the most suitable moisture content to which egg powder should be dried. These powders were stored at elevated temperatures in order to provide accelerated test conditions.

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Material selected from a thesis presented by one of us (W.H.W.) to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Issued as Paper No. 92 of the Canadian Committee on Food Preservation and as N.R.C. No. 1129.

[2] *Biochemist, Food Investigations.*

Materials and Procedure

In the initial experiment egg powder was obtained from a single Canadian producer. Melange consisting of a 1 : 1 mixture of Grades A and B fresh eggs was dried at inlet and outlet temperatures of 108° and 59° C. (227° and 138° F.), respectively. Separate portions of the powder were adjusted to moisture contents of 3.7, 5.2, and 8.5% by aeration with moistened air, and stored at temperatures of 7.1°, 15.6°, 23.8°, and 32.1° C. (45°, 60°, 75°, and 90° F.). Samples were removed for analysis after storage for 1, 2, 4, and 6 months at 7.1° to 23.8° C. and at semimonthly periods during 3.5 months at 32.1° C. Quality of the egg powders was assessed by determination of the fluorescence and potassium chloride values (2, 4). The fluorescence value has been shown to be related to flavour quality (2) whereas the potassium chloride value gives a measure of solubility and the condition of the fat–protein complex present (4, 5).

The above experiment gave information on the behaviour of powders containing various amounts of moisture during storage. However the number of moisture levels studied was insufficient to permit definite conclusions to be made concerning the most suitable moisture content to which egg powder should be dried.

In a second and more extensive experiment, egg powder was obtained from two representative Canadian plants and adjusted to six moisture levels. In Plant I the powder was prepared in a box type drier from a melange consisting of 2 : 1 mixture of storage and frozen eggs at inlet and outlet temperatures of 154° and 65° C., respectively (310° and 150° F.). In Plant II, melange consisting of a 1 : 1 mixture of storage and frozen eggs was dried in a cone type drier at inlet and outlet temperatures of 110° and 50° C., respectively (230° and 130° F.). In each instance the powder was taken directly from the drier and cooled promptly to about 0° C.

The moisture contents of the powders from the two plants were adjusted to approximately 2, 3, 4, 5, 6, and 7%. To achieve this, samples approximately 1100 gm. in weight, contained in large glass dishes, were placed in vacuum desiccators containing either anhydrous calcium chloride or the amount of water required to give the desired moisture content. The desiccators containing water were partially evacuated and closed off. Those containing calcium chloride were evacuated continuously with a Hyvac pump. All powders were thoroughly mixed at intervals of about two days, and samples removed for moisture analysis. After attaining the desired moisture content, the powders were thoroughly mixed and allowed to stand for one week to ensure uniform moisture distribution throughout the sample. These treatments were all made at 4.4° C. (40° F.) to minimize any change in quality.

Sixty-gram samples of the powders of each moisture content from each plant were placed in sealed tin cans, and heated in air ovens at temperatures of 26.8°, 34.9°, and 43.3° C. (80°, 95°, and 110° F.). Samples were removed for analysis after heating for 0.5, 1, 2, 3, 6, and 9 days at 34.9° and 43.3° C. and after 1, 3, 6, and 9 days at 26.8° C. Quality was assessed by measure-

ment of the fluorescence, potassium chloride, and refractometric values. The refractometric value is related to solubility (5). The significance of the other two measurements has been discussed previously.

Results

Initial Experiment

The importance of the various factors studied in affecting the fluorescence and potassium chloride values was assessed by analyses of variance. Since the sampling times employed at 32.1° C. differed from those at the lower temperatures, these data were treated separately. Moisture content and temperature and time of storage all had statistically significant effects on both the fluorescence and potassium chloride values (Table I).

TABLE I

Analyses of variance of the effect of moisture content on the fluorescence and potassium chloride values of dried egg powder stored at 7.1° to 32.1° C.

Storage temperature, ° C.	Source of variance	D.f.	Mean square	
			Fluorescence value	KCl value
7.1°, 15.6°, and 23.8° C.	Moisture content	2	1098**	2032**
	Temperature	2	2700**	3418**
	Time	3	740.1**	1332**
	Stored vs. non-stored	1	650.5**	1001**
	Moisture content × temperature	4	219.3**	5.298
	Moisture content × time	6	44.64**	41.13
	Temperature × time	6	137.9**	302.8*
	Residual	14	11.86	88.04
	Duplicate error	39	0.446	1.479
32.1° C.	Moisture content	2	2511**	844.1**
	Time	6	940.3**	356.4**
	Stored vs. non-stored	1	6998**	6976**
	Residual	14	90.19	24.30
	Duplicate error	24	1.23	0.8229

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

Fluorescence Values

Increases in the moisture content or the temperature or period of storage caused the mean fluorescence values to increase (Table II). The differences were greatest between moisture contents of 5.2 and 8.5%, temperatures of 15.6°, 23.8°, and 32.1° C. and storage periods of two to four months at 7.1° to 23.8° C. At 32.1° C. the mean fluorescence values changed rapidly during the first 1.5 months, and subsequently showed some tendency to decrease. This latter behaviour has been observed previously (6), although

at considerably higher fluorescence values, and was attributed to the decomposition of fluorescent materials.

TABLE II

MEAN VALUES FOR THE EFFECT OF MOISTURE CONTENT ON THE FLUORESCENCE AND POTASSIUM CHLORIDE VALUES OF DRIED EGG POWDER STORED AT 7.1° TO 32.1° C.

Moisture content

Moisture content, %	3.7	5.2	8.5
Mean fluorescence value[1]	28.6	31.2	40.9
Mean fluorescence value[2]	44.0	53.7	68.9
Mean KCl value[1]	66.9	58.7	49.3
Mean KCl value [2]	46.3	38.3	31.8

Temperature

Temperature, °C.	7.1	15.6	23.8	32.1
Mean fluorescence value[1, 2]	26.8	29.9	46.5	55.5
Mean KCl value[1, 2]	66.2	61.9	43.7	38.8

Storage period

Time, months	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	6.0
Mean fluorescence value[1]	23.6	—	27.7	—	30.3	—	—	—	38.4	41.2
Mean fluorescence value[2]	23.6	38.1	50.3	71.6	58.7	74.0	62.7	65.3	—	—
Mean KCl value[1]	70.7	—	69.2	—	57.8	—	—	—	51.8	50.3
Mean KCl value[2]	70.7	50.1	36.9	34.8	31.1	29.1	28.7	28.8	—	—

[1] *Mean values over all other conditions studied for powder stored at 7.1°, 15.6°, and 23.8° C.*
[2] *As for* [1], *but on powder stored at 32.1° C. only.*

The behaviour of the fluorescence values with variations in temperature or period of storage differed with the moisture content of the egg powder. The fluorescence values, averaged over all storage periods, of dried egg held at 7.1° or 15.6° C. and containing 3.7 or 5.2% moisture did not differ statistically amongst themselves, but were significantly less than that with 8.5% moisture, whereas at the higher storage temperatures the differences with moisture content were all significant. Similarly the fluorescence values of egg powder, averaged over temperatures of 7.1° to 23.8° C., showed relatively slow increase with time at moisture levels of 3.7 and 5.2%, whereas at 8.5% moisture the differences between all storage times were significant. At 32.1° C. the changes in the mean fluorescence values with time were rapid for all moisture contents, with the rate increasing with the moisture content.

Potassium Chloride Values

The mean potassium chloride values for each condition studied decreased with increase in moisture content or temperature or period of storage (Table II). Differences between the three moisture levels were all statistically significant and approximately of the same magnitude. On the average, an increase in temperature from 15.6° to 23.8° C. caused the greatest decrease in potassium chloride values. Differences with time were greatest between one and two months at 7.1° to 23.8° C., and during the first month of storage at 32.1° C. Subsequent changes were small. Thus, as noted previously for the fluorescence values, an increase in moisture content enhanced the rate of deterioration of dried egg powder during storage.

It is obvious from the above results that a storage temperature as low as 7.1° C. is not sufficient to prevent deterioration in dried egg if the moisture content of the powder is high. It would appear that the moisture content should preferably be as low as possible if satisfactory quality is to be maintained under the storage conditions studied.

Main Experiment

Data on the quality of the powders prior to heat treatment are given in Table III. Within experimental error the quality of the samples with various moisture contents from any one plant was the same. However, the overall quality of the egg powder obtained from Plant I was higher than that from Plant II. This difference is presumably due to variations in the predrying and drying practices employed in the two plants. While the moisture levels attained were not integral values they are, for the sake of convenience, referred to as such hereafter.

TABLE III

Initial measurements on dried egg powders secured from two Canadian plants and adjusted to various moisture contents

Adjusted moisture content, %		Fluorescence value		KCl value		Refractometric value	
Plant I	Plant II	Plant I	Plant II	Plant I	Plant II	Plant I	Plant II
2.20	1.97	14.9	17.2	78.2	79.6	269	255
3.00	2.98	15.0	17.3	78.6	77.9	267	251
3.93	3.97	14.8	16.9	76.4	82.1	267	257
4.99	4.90	15.0	16.2	76.9	77.9	267	254
5.76	5.86	14.6	17.1	75.2	76.5	265	254
6.90	6.91	14.6	16.5	75.9	77.9	264	252

Analyses of Variance

The relative importance of the various factors studied in affecting the fluorescence, potassium chloride, and refractometric values was assessed by means of analyses of variance. In making these computations, the data obtained for powders heated at 35.0° and 43.3° C. for 0.5 and 2 days were

disregarded since this information was not available at 26.7° C. The results show that temperature, time of heating, and moisture content usually had the greatest effect on quality, as assessed by the various measurements (Table IV). Variations attributable to differences between plants were of relatively minor importance as compared to the other factors, except for their effect on solubility as determined by the refractometric method.

TABLE IV

ANALYSES OF VARIANCE OF THE EFFECT OF MOISTURE CONTENT ON THE FLUORESCENCE, POTASSIUM CHLORIDE, AND REFRACTOMETRIC VALUES OF DRIED EGG POWDER STORED AT 26.8° TO 43.3° C.

Source of variance	D.f.	Mean square		
		Fluorescence value	KCl value	Refractometric value
Moisture content	5	1772**	1359**	4011**
Temperature	2	14,648**	14,159**	22,020**
Time	3	7715**	4720**	6891**
Plants	1	320.5**	167.1	6786**
Plants × moisture content	5	8.05	11.40	54.16
Plants × time	3	51.27	9.74	131.6
Plants × temperature	2	32.27	124.8	135.5
Moisture content × time	15	217.2**	141.1**	525.8**
Moisture content × temperature	10	383.9**	361.9**	1266**
Time × temperature	6	1471**	2263**	3920**
Residual	91	27.13	43.39	131.4
Duplicate error	144	0.3730	1.333	2.785

** *Indicates 1% level of statistical significance.*

Mean values for each variable, as averaged over all others for the entire experiment, are given for each measurement in Table V.

Regardless of the method of preparation, the keeping quality of dried egg varied directly with its moisture content and the temperature–time treatment to which it was subjected. Differences in quality were least between 2 and 3% moisture and usually greatest between 3, 4, and 5%. The greatest differences with temperature occurred between 34.9° and 43.3° C., which is in agreement with the results of a previous investigation (6). Changes with time in the mean potassium chloride and refractometric values were greatest between three and six days, whereas those in the fluorescence values were of approximately the same magnitude during each of the three-day periods studied. The differences between the mean values of the measurements for the two plants were small, and about the same magnitude as noted initially.

Details of the changes in the fluorescence, potassium chloride, and refractometric values treated in the analyses of variance are illustrated graphically in Figs. 1 to 3, respectively.

TABLE V

THE EFFECT OF MOISTURE CONTENT ON THE MEAN FLUORESCENCE, POTASSIUM CHLORIDE, AND REFRACTOMETRIC VALUES OF DRIED EGG POWDER STORED AT 26.8° TO 43.3° C.

Moisture content

Moisture content[2], %	2	3	4	5	6	7
Mean fluorescence value[1]	21.8	23.7	27.3	31.5	34.4	37.2
Mean KCl value[1]	74.7	71.8	68.7	65.7	63.0	60.7
Mean refractometric value[1]	264	263	257	250	246	241

Temperature

Temperature, ° C.	26.8	34.9	43.3
Mean fluorescence value[1]	18.8	26.5	42.8
Mean KCl value[1]	76.7	71.9	53.7
Mean refractometric value[1]	265	259	236

Storage period

Time, days	1	3	6	9
Mean fluorescence value[1]	17.0	25.5	33.8	40.9
Mean KCl value[1]	76.0	71.8	64.0	57.8
Mean refractometric value[1]	263	261	248	243

Plants

Plant	I	II
Mean fluorescence value[1]	28.2	30.3
Mean KCl value[1]	68.2	66.7
Mean refractometric value[1]	258	248

[1] *As averaged over all other conditions for the whole experiment.*

[2] *Approximate moisture contents, see Table III for actual values.*

Fluorescence Value

An increase in the temperature, time of heating, or moisture content caused an increase in the fluorescence values (Fig. 1). Changes in the fluorescence value with time depended to a considerable extent on the moisture content of the powder and the temperature at which it was heated. At 26.7° C. the relation was essentially linear, the slope of the curve increasing with increase in the moisture content. However, at 35.0° and 43.3° C. there was considerable difference in behaviour between the various moisture levels. At 35.0° C. the fluorescence values of powders containing 5% or more moisture usually increased slowly during the first three days and rapidly subsequently, whereas at 43.3° C. the opposite behaviour occurred. At the lower moisture

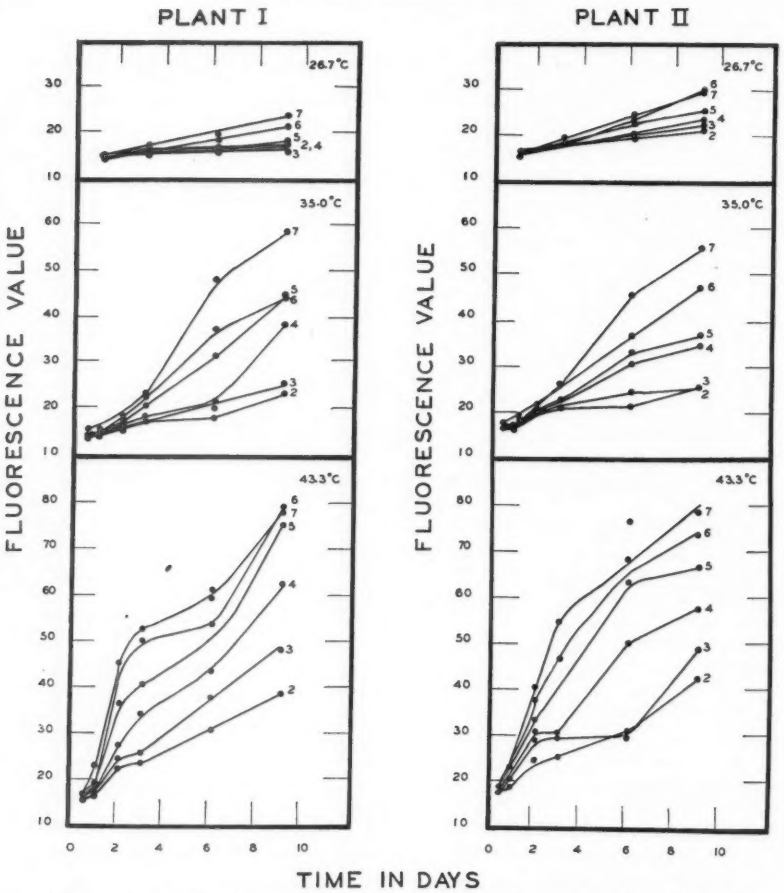

FIG. 1. *Effect of moisture contents of 2 to 7% on the fluorescence values of dried egg powders.*

levels of 2 or 3% the rate of increase of the fluorescence values was usually less after three days at both temperatures.

Variations in the moisture content and the temperature and time of heating had approximately the same effect on the fluorescence values regardless of the plant in which the dried egg was manufactured. This suggests that the differences in quality normally observed between powders from Canadian plants are due primarily to variations in heat treatment and moisture content.

Potassium Chloride Value

The general behaviour of the potassium chloride values with variations in the moisture content, temperature, time of heating, and manufacture of the

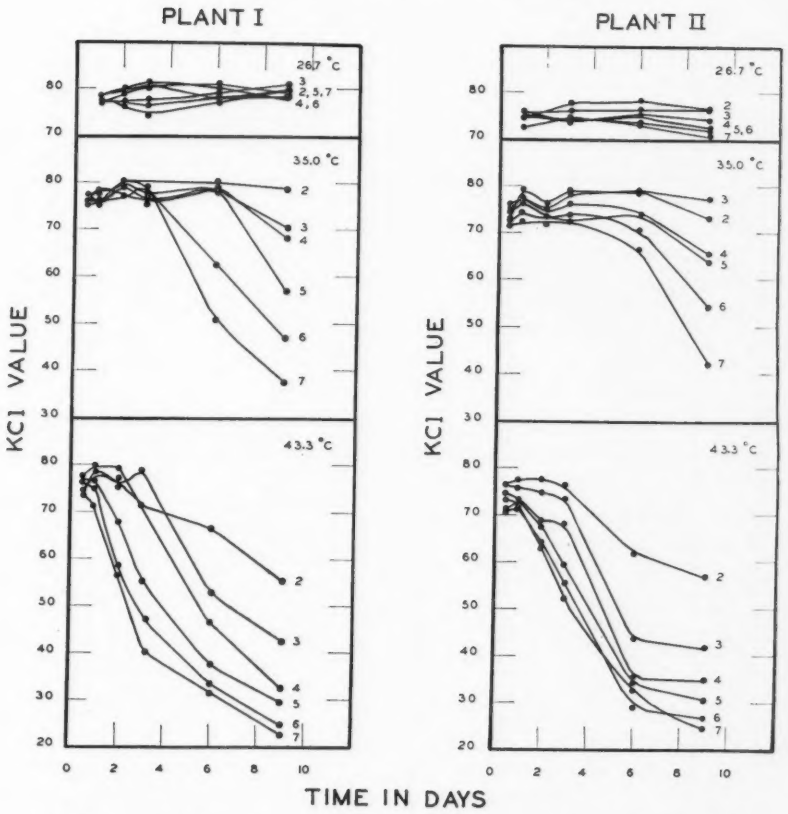

FIG. 2. *Effect of moisture contents of 2 to 7% on the potassium chloride values of dried egg powders.*

powder was usually similar to that noted above for the fluorescence value, except that the trend of the changes was in the opposite direction (Fig. 2). At 26.7° C. the changes in potassium chloride value were usually small and somewhat variable. There was, however, definite indication that the higher moisture contents were associated with lower potassium chloride values. At the higher temperatures the rate of decrease of the potassium chloride values was greater the higher the moisture content and temperature.

Refractometric Values

The refractometric values decreased with increase in the moisture content and temperature and time of heating (Fig. 3). The results obtained at 26.7°C. and 35.0° C. were somewhat variable. Nevertheless, there was a definite

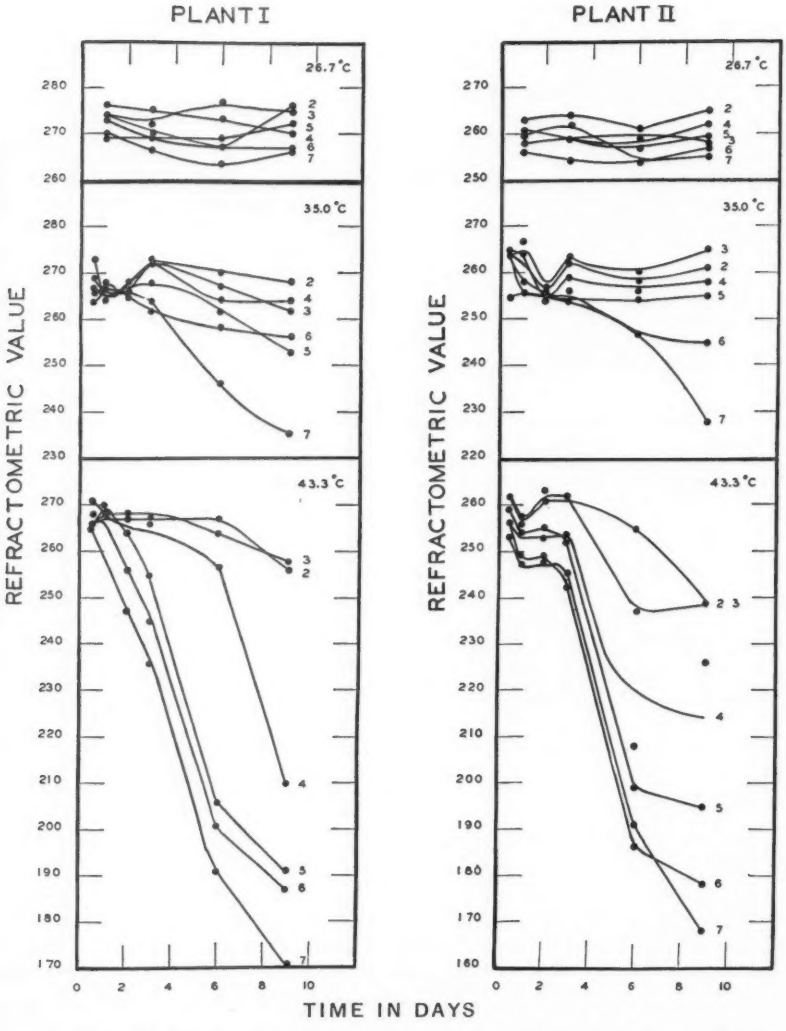

FIG. 3. *Effect of moisture contents of 2 to 7% on the refractometric values of dried egg powders.*

tendency for the refractometric values to decrease more rapidly with time at the higher moisture levels. At 43.3° C. changes in the solubility of powders containing 2 or 3% moisture were relatively slow and similar in character, whereas at the higher moisture levels the solubility usually decreased markedly between three and six days, and more slowly subsequently.

Interrelation of Methods for Assessing Quality

The degree of interrelation of the fluorescence, potassium chloride, and refractometric values was determined by the computation of simple coefficients of correlation. Data were used only for those samples that corresponded to egg powder of quality suitable for export by present Canadian standards, i.e. a fluorescence value of 52 or lower and a potassium chloride value of 40 or higher. It is to be noted that such a selection includes a higher proportion of samples of poor quality than is normally encountered in Canadian dried egg powders. All three methods were significantly interrelated (Table VI). The relative order of the magnitudes of the correlation coefficients is that expected when it is recalled that, on the basis of present knowledge, the fluorescence value is related to overall quality, the potassium value to both overall quality and solubility and the refractometric value to solubility alone.

TABLE VI

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE FLUORESCENCE, POTASSIUM CHLORIDE, AND REFRACTOMETRIC VALUES OF DRIED EGG POWDER

Method	Method	
	KCl value	Refractometric value
Fluorescence value	−0.88**	−0.71**
KCl value	—	0.75**

** *Indicates 1% level of statistical significance for the 127 degrees of freedom available.*

Conclusion and Discussion

The major portion of the variations in quality of egg powders studied was attributable to moisture content and the temperature and time of heating. Differences in the constituents of the egg melange and in predrying and drying practices employed in the two plants were of less importance. Since at all temperatures from 7.1° to 43.3° C. deterioration occurred in powder containing as little as 2 to 3% moisture, it appears that there is no limit of moisture content, commercially attainable at present, below which deterioration can be prevented regardless of the temperature to which dried egg is exposed.

The importance of moisture content on keeping quality may be further demonstrated by interpreting the experimental data in terms of the time required for egg powders, of different moisture contents and held at various temperatures, to deteriorate to the lowest quality acceptable for export to England as first grade powders, namely, a fluorescence value of 26. For example, at 7.1° C. such periods for powders containing about 3, 5, and 7% moisture were approximately 3, 3, and 1.5 months, respectively; at 26.8° C., 17, 12, and 6 days; and at 43.3° C., 46, 34, and 26 hours, respectively.

On the basis of this investigation it is now required that the moisture content of all egg powder prepared for export to England shall be as low as

is compatible with the preparation of a powder of satisfactory quality and in no circumstance greater than 5%. While it is obvious that the moisture content should preferably be 2% or lower, the attainment of these levels would probably result in powders of undesirably low initial quality because of the higher drying temperatures usually required by present Canadian drying practice.

Acknowledgments

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